



## Nitrification biokinetics in rapid sand filters for drinking water treatment

Tatari, Karolina

*Publication date:*  
2014

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Tatari, K. (2014). *Nitrification biokinetics in rapid sand filters for drinking water treatment*. DTU Environment.

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Nitrification biokinetics in rapid sand filters for drinking water treatment



**Karolina Tatari**



# Nitrification biokinetics in rapid sand filters for drinking water treatment

Karolina Tatari

PhD Thesis  
March 2014

DTU Environment  
Department of Environmental Engineering  
Technical University of Denmark

**Karolina Tatari**

**Nitrification biokinetics in rapid sand filters for drinking water treatment**

PhD Thesis, March 2014

The synopsis part of this thesis is available as a pdf-file for download from the DTU research database ORBIT: <http://www.orbit.dtu.dk>

Address: DTU Environment  
Department of Environmental Engineering  
Technical University of Denmark  
Miljoevej, building 113  
2800 Kgs. Lyngby  
Denmark

Phone reception: +45 4525 1600

Fax: +45 4593 2850

Homepage: <http://www.env.dtu.dk>

E-mail: [reception@env.dtu.dk](mailto:reception@env.dtu.dk)

Printed by: Vester Kopi  
March 2014

Cover: Torben Dolin

# Preface

This thesis presents the research carried out as a PhD project at DTU Environment, Technical University of Denmark (DTU) from 2010 to 2013. The project was founded by the Danish Council for Strategic Research via project DW-Biofilters and DTU. The supervisors were Hans-Jørgen Albrechtsen and Barth F. Smets from DTU Environment, and Peter B. Nielsen from Krüger A/S.

The PhD thesis comprises a synopsis of the work presented in one published paper, two submitted papers and one manuscript in preparation. In the synopsis the papers are referred to by the author names and the roman numerals (e.g. Tatari et al., I). The included papers are:

- I.** Tatari, K., Smets, B. F., Albrechtsen H.-J., 2013. A novel bench-scale column assay to investigate site-specific nitrification biokinetics in biological rapid sand filters. *Water Research* 2013, 47, (16), 6380-6387.
- II.** Tatari, K. Musovic, S., Smets, B. F., Albrechtsen, H.-J. Strong stratification in nitrifier activity, density and type in rapid sand filters treating drinking water. Submitted to *Environmental Science & Technology*.
- III.** Tatari, K., Gülay, A., Thamdrup, B., Albrechtsen, H.-J., Smets, B. F. Use and challenges of specific nitrification inhibitors applied to groundwater treatment biofilters. Submitted to *Water Research*.
- IV.** Gülay, A., Tatari, K., Musovic, S., Mateiu, R. V., Albrechtsen, H.-J., Smets, B. F. Internal mineral porosity supports microbial activity in stratified rapid sand filters for drinking water treatment. In preparation for *Environmental Microbiology*.

In this online version of the thesis, the papers are not included but can be obtained from electronic article databases e.g. via [www.orbit.dtu.dk](http://www.orbit.dtu.dk) or on request from:

DTU Environment  
Technical University of Denmark  
Miljøvej, Building 113  
2800 Kgs. Lyngby  
Denmark

[reception@env.dtu.dk](mailto:reception@env.dtu.dk)

This PhD work has contributed also to the following manuscript in preparation, and has produced three extended abstracts from dissemination at international conferences:

Wagner F. B., Lee C. O., Tatari K., Nielsen P. B., Albrechtsen H.-J. Nutrient limitations in drinking water rapid sandfilters with incomplete ammonium removal. In preparation for Water Research.

Tatari, K., Smets, B. F., Nielsen, P. B., Lind, S., Albrechtsen, H.-J. 2012. Effect of surface loading fluctuations in rapid sand filters used to remove ammonium from drinking water. Water Quality and Technology Conference and Exposition (WQTC), Toronto, Canada. Conference proceedings.

Tatari, K., Gudbrand, T., Smets, B. F., Lind, S., Albrechtsen, H.-J. 2013 Nitrification activity stratifies in a rapid sand filter for drinking water treatment - A study in two Danish waterworks. 7<sup>th</sup> Annual Meeting Danish Water Research and Innovation Platform (DWRIP), Kgs. Lyngby. Abstract Proceedings.

Tatari, K., Musovic, S., Smets, B. F., Nielsen, P. B., Lind, S., Albrechtsen, H.-J. 2013. Stratification of nitrification activity in rapid sand filters for drinking water treatment. Biological Treatment Symposium, Denver, Colorado. Conference proceedings.

Tatari, K., Lee, C. O., Smets, B. F., Nielsen, P. B., Albrechtsen, H.-J. 2013. Examining biological rapid sand filters for drinking water treatment as biofilm reactors: experimental and modeling approach. 9<sup>th</sup> International Conference on Biofilm Reactors, Paris, France. Conference proceedings

# Acknowledgments

First and foremost, I would like to thank my two main supervisors, Hans-Jørgen Albrechtsen and Barth F. Smets for their guidance and support throughout the entire PhD project. Thanks also to Peter B. Nielsen for providing all needed information and contacts with the waterworks involved in this study. The waterworks involved in this work are greatly acknowledged for allowing us to sample and investigate their filters. George Amoako is particularly acknowledged for his availability and patience with us messing up in the filters.

A warm thank to Arda Gülay for the great collaboration and fun working together. Thanks also to my office mate-ven Sanin Musovic and to Carson Lee for their help and fruitful discussions during the entire PhD project. Special thanks go to Mathilde Hedegaard for the Danish version of the abstract and to Florian Wagner for his always prompt feedback and long discussions. Thanks also to Katie Lin for the great time during external courses and conferences.

Many thanks to Bo Thamdrup for the collaboration and support in the laboratory analysis at SDU. Lab technicians, and especially Hector Diaz and Sinh Nguyen are acknowledged for their help and availability.

Very special thanks go to my friend Elena for her patience and help in formatting and proofreading parts of this thesis. A huge hug to all my friends and especially Katerina, Tina, Nassia, Nemanja, Suzi, Filippo and Marco, and of course to Davide for always making me smile.

A grateful thank to my family, who has always been there for me with a lot of love. And especially to my father who has also always been there.



# Abstract

Anoxic groundwater is widely used as a drinking water source. Treatment usually consists of aeration, where oxygen is introduced in the water, and rapid sand filtration that removes unwanted compounds to comply with drinking water quality standards. Rapid sand filters are often the only removal barrier for particulate matter and dissolved components before the distribution network.  $\text{NH}_4^+$ ,  $\text{Fe}^{+2}$  and  $\text{Mn}^{+2}$  are the main compounds removed during filtration by biological or chemical oxidation. Nitrification is a biological process, driven by autotrophic microorganisms that use the  $\text{NH}_4^+$  in the water as substrate for their growth. Although nitrification is well documented for several environmental systems such as activated sludge or soil, limited knowledge is available for drinking water biofilters. As a result, rapid sand filters are typically designed and operated based on rules of thumb and operator's experience. Empirical design and operation parameters include filter depth, average loading rates as well as backwashing strategy and frequency.

Filter operation becomes particularly challenging in poor performing filters, where effluent  $\text{NH}_4^+$  concentration exceeds the guideline limits. In these cases, tentative remedial solutions are adopted that can be expensive, time consuming and may not always resolve the problem. Nitrification insight is therefore needed, not only to identify possible causes of poor filter performance, but mainly to prevent the occurrence of such cases. This PhD project aimed to provide insight into nitrification biokinetics as a basis for filter design and operation optimization.

Primary information about the loading and the removal rate in a filter can be obtained from the  $\text{NH}_4^+$  influent and effluent concentrations. However, depth profiling can identify where nitrification takes place in a filter and if the entire filter depth is active. Depth profiling in a well performing filter (effluent  $< 0.05 \text{ mg NH}_4^+\text{-N /L}$ ) at Islevbro waterworks showed that  $\text{NH}_4^+$  was already removed at 0.20 m depth, while no  $\text{NH}_4^+$  was detected in the remaining 0.50 m of the filter. To investigate the nitrification activity of these deeper filter layers, we developed a lab-scale assay that allowed modification of the loading conditions.

The assay consisted of a small column packed with filter material, where the  $\text{NH}_4^+$  loading level was controlled. The novelty of the assay compared with existing methods was that the biomass was not detached from the filter material and the biofilm integrity was maintained.

The assay was used to investigate stratification of nitrification activity with depth in filters performing differently. Nitrification activity in the Islevbro filter was strongly stratified and was highest in the top layer, although deeper layers were also active when exposed to substantial  $\text{NH}_4^+$  loadings. In a poor performing filter (average effluent  $0.1 \text{ mg NH}_4^+\text{-N/L}$ ), low nitrification activity was detected at all depths and no stratification was observed.

The strong stratification pattern observed in the Islevbro filter was explained by the presence of mineral coating on the sand grains. Mineral coating was predominantly present in the top filter layer, making the sand grains lighter and dictating their distribution with depth. Mineral coating was positively correlated with internal porosity and higher nitrification activity. The latter correlation was attributed to a larger surface area in sand grains with more mineral coating.

Nitrification kinetics in the Islevbro filter was investigated with depth and was interpreted based on the abundance of active nitrifying microorganisms. Ammonium Oxidizing Bacteria (AOB) abundance stratified consistently with the distribution of nitrification activity. Ammonium Oxidizing Archaea (AOA) were detected in the same order of magnitude as AOB, but did not follow any stratification pattern.

Nitrification kinetics described by a 1-D biofilm model, estimated different AOB half saturation concentrations and maximum growth rates with depth. Biokinetic behavior also varied with depth, as top and middle layer showed an up-shift nitrification capacity and an increase in the maximum growth rate with loading increase. This behavior was not observed in the bottom filter layer. The maximum cell specific activity of AOB was higher in the top and middle filter layers compared with the bottom, suggesting that different AOB types were active in each depth.

All in all, this work quantified the depth specific nitrification rates and identified the occurrence of activity, density and active species stratification with depth in rapid sand filters. Stratification may or may not be desired in a filter, depending on influent water quality and effluent water requirements.

Although stratification may decrease filter efficiency due to inactive filter zones, this may stimulate  $\text{Mn}^{+2}$  removal. Besides that, stratification may select for nitrifiers with high affinities in the deeper filter layers that remove  $\text{NH}_4^+$  up to very low concentrations. Additionally, loading rate is also a determining parameter for filter performance, and a safe-operational loading window that uses efficiently the filter volume but does not exceed the maximum capacity needs to be determined.

# Dansk sammenfatning

Anaerobt grundvand er en udbredt drikkevandsressource. Før vandet lever op til drikkevandskvalitetsstandarderne kommer det igennem en behandlingsproces der består af beluftning, hvor ilt tilføres, hvorefter vandet filtreres i sandfiltre, der fjerner uønskede stoffer ned til acceptable koncentrationer. Således er sandfiltrering ofte den eneste barriere, der fjerner partikler og opløste stoffer før vandet ledes ud i distributionsnettet.

De stoffer, der primært fjernes ved filtreringen, er  $\text{NH}_4^+$ ,  $\text{Fe}^{+2}$  og  $\text{Mn}^{+2}$ . Disse fjernes alle ved biologisk eller kemisk oxidering. Nitrifikation er en biologisk proces, hvor autotrofe mikroorganismer anvender  $\text{NH}_4^+$  som væksts substrat. Nitrifikation er en veldokumenteret proces i andre teknologiske og naturlige systemer, så som aktivt slam og jord, men på trods af det, vides der forsat ikke meget om processen i biologiske filtre i drikkevandsbehandlingen. Dette resulterer i, at design og drift af sandfiltre typisk er baseret på tommefingerregler og operatørens erfaring. Empiriske design- og driftsparametre omfatter bl.a. filterdybde, gennemsnitlig belastning samt strategi og hyppighed af returskylning.

Driften er særligt udfordrende i filtre, der ikke fungerer optimalt, og hvor udløbskoncentrationen af  $\text{NH}_4^+$  overskrider retningslinjerne. I disse tilfælde bliver forsøgsvisse løsninger taget i brug for at afhjælpe problemet, hvilket kan være en dyr og tidskrævende proces, der ikke altid medfører en endelig løsning. Det er derfor nødvendigt med en bedre indsigt i nitrifikationen for at kunne identificere mulige årsager til at nogle filtre ikke fungerer optimalt, og især for at kunne forhindre at disse tilfælde opstår. Formålet med dette PhD projekt var at opnå indsigt i nitrifikationens biokinetik og dermed skabe et grundlag for optimering af filter design og drift.

Koncentration af  $\text{NH}_4^+$  i filterets indløbs- og udløbsvand kan give information om filterets belastning og fjernelsesrate. Dybdeprofiler kan anvendes til at identificere, hvor i filteret nitrifikationen sker, og om der er aktivitet i alle dybder. I et filter på Islevbro vandværk, der levede op til standarderne (udløbskoncentration  $< 0.05 \text{ mg NH}_4^+ \text{-N /L}$ ), viste dybdeprofiler at  $\text{NH}_4^+$  allerede var fjernet i 0.20 meters dybde. For at undersøge nitrifikationen i de dybere filterlag, udviklede vi en laboratoriekolonne, hvori belastningen af  $\text{NH}_4^+$  kunne ændres.

Laboratoriekolonnen bestod af en lille kolonne med filtermateriale, hvor belastningen af  $\text{NH}_4^+$  i indløbet kunne kontrolleres. Denne opstilling adskiller sig fra eksisterende metoder ved, at biomassen ikke rives løs fra filtermaterialet og biofilmen bevares som en helhed.

Opstillingen blev anvendt til at undersøge filtre, der fungerede forskelligt, og det blev undersøgt hvordan nitrifikationsaktiviteten var i forskellige dybder i filteret. I et filter på Islevbro var nitrifikationsaktiviteten var meget lagdelt. Aktiviteten var højest i toppen af filteret. De dybere lag var også aktive, når de blev udsat for en høj belastning af  $\text{NH}_4^+$ . I et filter der ikke levede op til standarderne (gennemsnitlig udløbskoncentration  $0.1 \text{ mg NH}_4^+\text{-N/L}$ ), var nitrifikationsaktiviteten lav i alle filterdybder, og der blev ikke observeret nogen lagdeling i aktiviteten.

Den tydelige lagdeling i filteret på Islevbro skyldes en mineralcoating på sandkornene. Mineralcoatingen blev primært fundet i det øverste filterlag og gjorde sandkornene lettere, hvilket påvirkede kornenes fordeling i filteret. Nitrifikationsaktiviteten og den indvendige porøsitet korrelerede positivt med mineralcoatingen, hvilket blev forklaret ved, at sandkorn med mineralcoating havde et større overfladeareal.

Den horisontale lagdeling i nitrifikationskinetikken af filteret på Islevbro, blev undersøgt og fortolket i forhold til tilstedeværelsen af aktive nitrificerende mikroorganismer. Tilstedeværelsen af ammonium oxiderende bakterier (AOB) var lagdelt, og fulgte fordelingen af nitrifikationsaktiviteten. Ammonium oxiderende archaea (AOA) blev målt til at være i same størrelsesorden som AOB, men var ikke lagdelt.

Nitrifikationskinetikken blev beskrevet af en 1-D biofilm model, og blev anvendt til at estimere halvmætningskonstant og maksimale vækstrater af AOB i de forskellige lag i sandfilteret. Biokinetikken varierede også mellem lagene - det øverste og mellemste lag kunne øge nitrifikationskapaciteten og den maksimale vækstrate når belastningen blev forøget, hvilket ikke gjorde sig gældende for det nederste filterlag. Den maksimale celledensitet for AOB var større i det øverste og mellemste lag i filteret end i det nederste, hvilket indikerer, at forskellige typer af AOB var aktive i de respektive lag.

Sammenfattende har projektet kvantificeret den dybde-specifikke nitrifikationsrate og identificeret fordelingen af aktivitet, koncentration og aktive grupper i sandfilterets lag. Stratificering i filteret kan være hensigtsmæssig eller uønsket, afhængigt af indløbsvandets kvalitet og kravene til det behandlede vand.

Selv om stratificering muligvis nedsætter filtereffektiviteten på grund af inaktive lag i filtret kan dette på den anden side måske stimulere  $\text{Mn}^{+2}$ -fjernelsen. Desuden giver stratificeringen mulighed for selektion af nitrifikanter med høj affinitet i filtrets dybere lag, og dermed mulighed for at fjerne  $\text{NH}_4^+$  ned til meget lave koncentrationer. Belastningen er også en afgørende parameter for præstationen af filteret, og det er derfor nødvendigt at bestemme et sikkert belastningsinterval, hvori filtervolumen udnyttes effektivt uden at den maksimale kapacitet overskrides.

# Table of contents

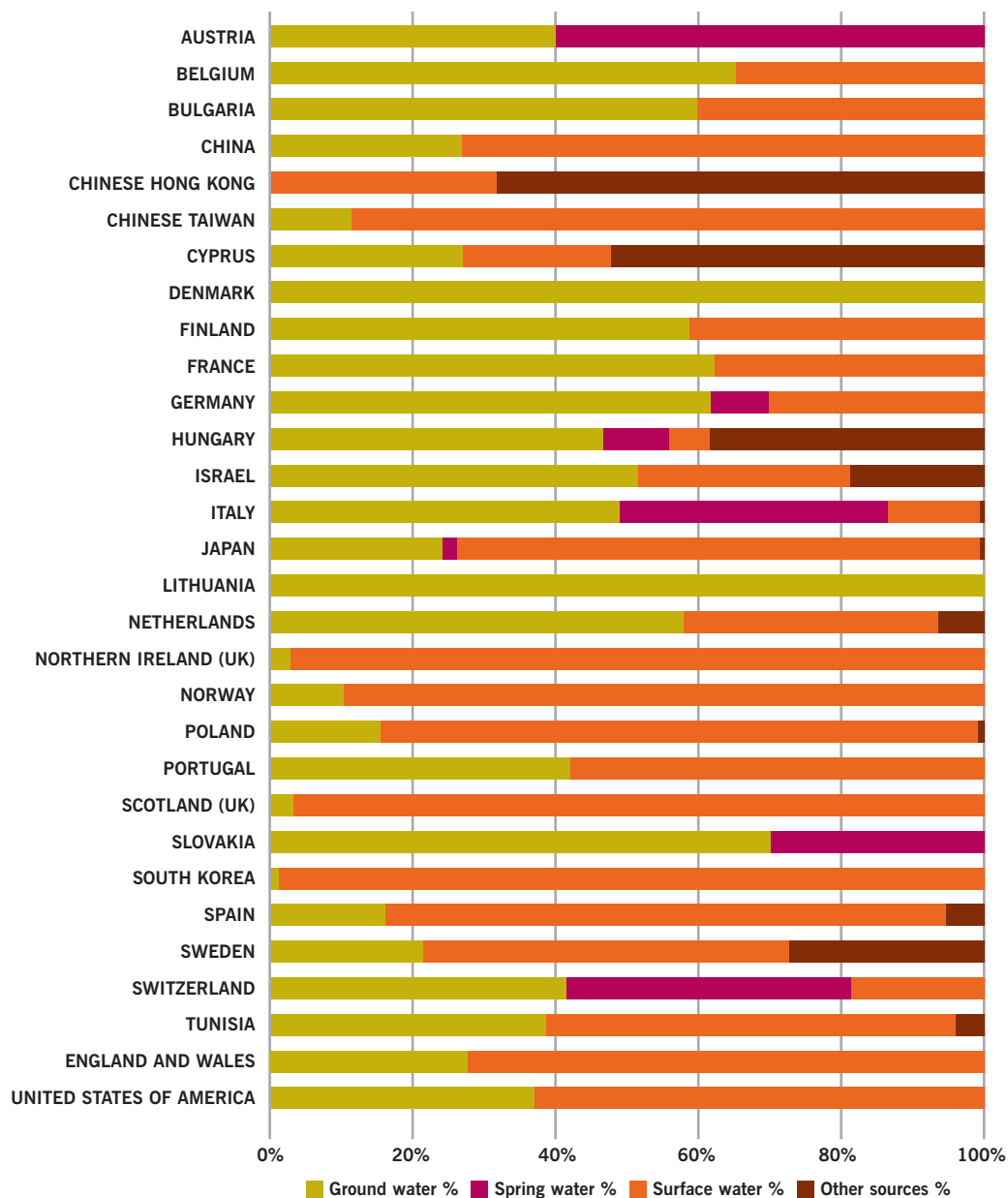
<b>Preface .....</b>	<b>i</b>
<b>Acknowledgments.....</b>	<b>iii</b>
<b>Abstract .....</b>	<b>iv</b>
<b>Dansk sammenfatning.....</b>	<b>vi</b>
<b>Table of contents .....</b>	<b>ix</b>
<b>1. Introduction.....</b>	<b>1</b>
1.1. Groundwater as a drinking water source .....	1
1.2. Typical waterworks configuration.....	3
1.3. Granular filter types.....	4
1.4. Rapid sand filtration .....	5
1.5. Removal of dissolved compounds in rapid sand filters.....	6
1.6. Problems in rapid sand filters .....	7
1.7. Motivation of the project.....	7
1.8. Objectives .....	9
<b>2. Methods used to investigate nitrification biokinetics .....</b>	<b>11</b>
2.1. Importance of scale in nitrification biokinetic investigations .....	11
2.2. Nitrification investigations in full-scale filters.....	12
2.2.1. $\text{NH}_4^+$ removal in full-scale filters .....	12
2.2.2. $\text{NH}_4^+$ depth profiling.....	13
2.3. Nitrification biokinetics in bench-scale assays .....	15
2.3.1. Types of bench-scale assays.....	15
2.3.2. Principles & use of the developed bench-scale assay .....	16
2.4. Mathematical modeling of nitrification kinetics in the bench-scale assay.....	18
<b>3. Stratification of nitrification activity with depth.....</b>	<b>23</b>
3.1. Distribution of nitrification activity with depth in different filters .....	23
3.2. Link of nitrification activity stratification with filter performance.....	25
3.3. Mineral coating properties and effect on stratification .....	26
3.4. Correlation of nitrification activity with mineral coating presence and surface area .....	28

<b>4. Site-specific nitrification kinetics and physiology in rapid sand-filters</b>	<b>31</b>
4.1. Active ammonium oxidizing species .....	31
4.2. Nitrification kinetics at different loading conditions .....	32
4.3. External mass transfer limitations in rapid sand filters .....	33
4.4. Cell specific nitrification activity and physiology of active nitrifying species	35
4.5. Selective inhibition of $\text{NH}_4^+$ and $\text{NO}_2^-$ oxidation-challenges with the use of ATU and $\text{ClO}_3^-$ .....	36
<b>5. Conclusions.....</b>	<b>41</b>
<b>6. Practical considerations &amp; perspectives .....</b>	<b>43</b>
<b>7. References.....</b>	<b>45</b>
<b>8. Papers.....</b>	<b>51</b>

# 1. Introduction

## 1.1. Groundwater as a drinking water source

Groundwater and surface water are worldwide the most common sources used for drinking water production (Figure 1). Water supply in several North European countries and partly in the United States relies on groundwater sources. In Denmark, where this study was conducted, groundwater is the only water source used.



**Figure 1.** Worldwide abstraction sources for drinking water supply in 2008 (IWA, 2010)



The water source used dictates the main raw water quality characteristics and therefore the treatment needed to meet drinking water quality standards in the supplied water. The main difference between groundwater and surface water is that the first is typically anoxic, while the latter is aerobic. Anoxic conditions in the aquifer result in the presence of reduced compounds in the raw water, such as  $\text{NH}_4^+$ ,  $\text{Fe}^{+2}$ ,  $\text{Mn}^{+2}$ ,  $\text{CH}_4$  and  $\text{H}_2\text{S}$ . Organic carbon content is normally low, contrarily with typical surface waters. Surface water on the other hand contains more particulate components and organic material, thus requiring treatment in several steps. Although groundwater quality depends on aquifer characteristics, Table 1 illustrates typical concentrations of inorganic compounds present in Danish groundwater.

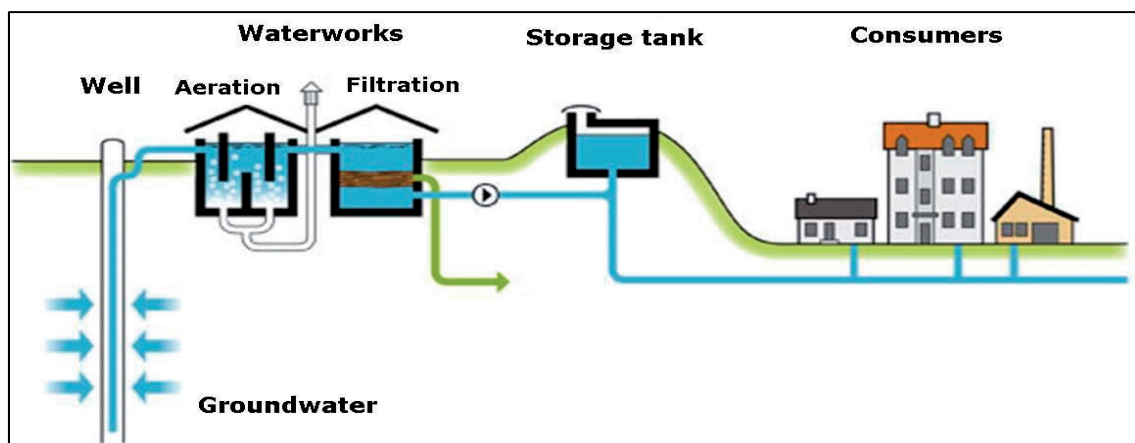
**Table 1.** Typical range of selected inorganic compounds present in Danish groundwater. Reported values are rounded from Eberling, 2002

Compound (mg/L)	Low	High
$\text{HCO}_3^-$	10	500
$\text{Ca}^{+2}$	10	200
$\text{NO}_3^-$	0.5	200
$\text{SO}_4^{2-}$	20	100
$\text{Cl}^-$	30	80
$\text{Mg}^{+2}$	2	40
$\text{K}^{+1}$	2	10
$\text{NH}_4^+$	0.1	7
$\text{Fe}^{+2}$	0.02	5
$\text{Mn}^{+2}$	0.001	3

Concentrations of  $\text{NH}_4^+$ ,  $\text{Fe}^{+2}$  and  $\text{Mn}^{+2}$  in the effluent water are required to comply with guideline values. Maximum allowed concentrations in Europe are 0.5 mg/L for  $\text{NH}_4^+$ , 0.2 mg/L for  $\text{Fe}^{+2}$  and 0.05 mg/L for  $\text{Mn}^{+2}$  (Council Directive 98/93/EC, 1998). A stricter  $\text{NH}_4^+$  guideline limit of 0.05 mg/L is applied in Denmark (Miljøministeriet, 2011), as no chlorine is used for disinfection in the distribution system. Residual  $\text{NH}_4^+$  after treatment can therefore support microbial growth (Chu et al., 2005), oxygen consumption in the pipes (Zhang et al., 2009) and accumulation of toxic  $\text{NO}_2^-$  from incomplete  $\text{NH}_4^+$  oxidation (Lytle et al., 2007). Dissolved  $\text{O}_2$  and  $\text{NO}_2^-$  concentrations are also regulated at the waterworks effluent to 5 and 0.01 mg/L respectively (Miljøministeriet, 2011).

## 1.2. Typical waterworks configuration

Groundwater is usually abstracted from several well fields and is mixed before entering the waterworks. A typical series of treatment steps employed at the waterworks is illustrated in Figure 2. Aeration introduces oxygen in the anoxic groundwater and can stimulate stripping of gaseous contaminants potentially present in the water e.g.  $\text{H}_2\text{S}$  and  $\text{CH}_4$ . Several aeration configurations may be used, including cascade aeration, diffuser aeration or oxygenation (Winther et al., 2010). The introduced oxygen causes oxidation of the reduced metal species i.e.  $\text{Fe}^{+2}$  and  $\text{Mn}^{+2}$  to  $\text{Fe}^{+3}$  and  $\text{Mn}^{+4}$ . To facilitate the removal of these metals, aeration is often followed by a reaction time that provides extra time for oxidation and precipitation of their hydroxydes.



**Figure 2.** Typical water supply system in Denmark includes groundwater abstraction, treatment at the waterworks and supply to the consumers (Vandcentersyd, 2013)

Filtration follows aeration and aims to remove particulate components and pathogens (AWWA, 1999), as well as  $\text{NH}_4^+$ ,  $\text{Fe}^{+2}$  and  $\text{Mn}^{+2}$ . Filtration is discussed in details in the next section. After filtration, the water is stored in simple storage or elevated tanks before supply to the distribution network.

### 1.3. Granular filter types

Granular filters have been in use for several decades and different technologies have been employed in groundwater treatment, among which slow and rapid sand filtration are the most common. The two filter types differ by principle in the hydraulic loading rate, and in several other design and operation parameters as summarized in Table 2. Rapid sand filtration has widely replaced slow sand filtration because of higher removal efficiencies and smaller footprint required (Crittenden et al., 2005).

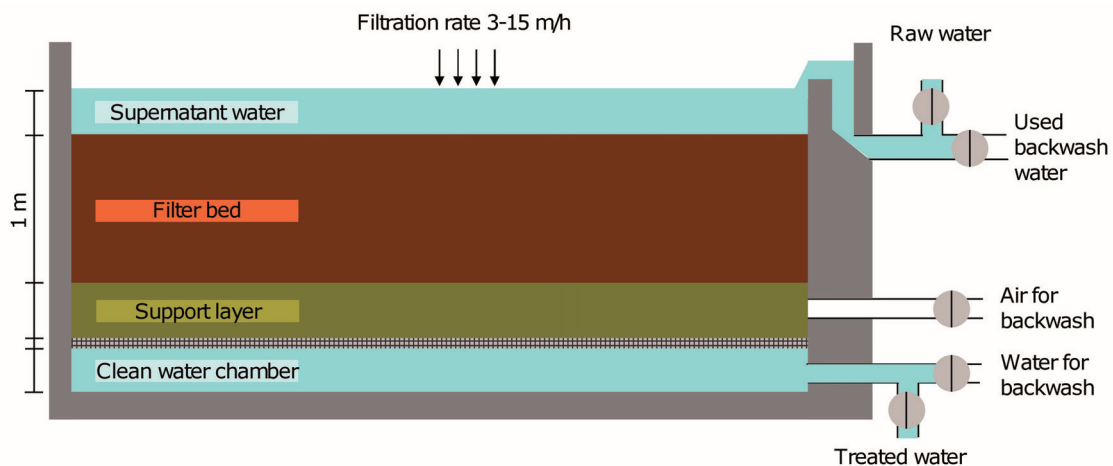
**Table 2.** Comparison between typical ranges of design and operational parameters in slow and rapid sand filtration (Adapted from Crittenden et al., 2005)<sup>a</sup>

Process characteristic	Slow sand filtration	Rapid sand filtration
Hydraulic loading rate (m/h)	0.05-0.20	3.0-15
Filter media diameter (mm)	0.30-0.45	0.50-1.2
Bed depth (m)	0.9-1.5	0.6-1.8
Required head (m)	0.9-1.5	1.8-3.0
Filtering cycle length	1-6 months	1-20 days
Ripening period	Several days	15 min- 2 h
Cleaning method	Scraping	Backwashing

<sup>a</sup>Values represent typical ranges and some filters are designed and operated outside these ranges

## 1.4. Rapid sand filtration

Rapid sand filters may consist of a single sand, or a of dual media layer of anthracite and sand. Dual media filters are deeper, with total bed depths of 1.5-1.8 m, supported by a garnet or ilmenite layer at the bottom (Lopato, 2011). However, dual media filtration is gradually abandoned, as single media filters appear to be more efficient in operation and backwashing. Water flows in the filters normally by gravity, although few designs use pressurized flow instead. Single media filters follow in general the configuration illustrated in Figure 3. Typical sand bed depth ranges between 0.6 and 1 m, with a 0.1 m gravel supporting layer. Due to the relatively shallow filter bed, pre- and after- filtration steps may be employed depending on the water type. In this case, pre-filters aim to remove mainly  $\text{Fe}^{+2}$  and have a bed of coarser material, such as gravel or stones of 20-80 mm size. After-filters have a bed of finer quartz sand particles of nominal size typically between 0.6 and 1.2 mm.



**Figure 3.** Typical configuration of single media gravity sand filters (Lopato, 2011)

Filters are backwashed every 4-15 days by high water up-flows, often combined with air scouring (AWWA, 1999). Backwashing partially lifts and mixes the filter material to remove particulate matter, excess biomass and inorganic deposits settling on the filter bed.

## 1.5. Removal of dissolved compounds in rapid sand filters

Filters are often the only removal barrier during groundwater treatment for particulate and dissolved compounds. Particulate matter is removed by physical straining (AWWA, 1999), whereas dissolved components are removed by chemical or biological processes.

$\text{Fe}^{+2}$  is chemically oxidized by different mechanisms during filtration, depending on the water quality and the operation characteristics. Homogeneous oxidation consists of oxidation to  $\text{Fe}^{+3}$  and hydrolysis in the bulk phase, which generates flocs that are physically retained in the filter (Sharma et al., 2005). Alternatively,  $\text{Fe}^{+2}$  may adsorb on the already deposited  $\text{Fe}^{+3}$  on the filter material, and oxidize there forming a new iron oxide layer (heterogeneous oxidation) (Sharma et al., 2005). Alkalinity as  $\text{HCO}_3^-$  is consumed by chemical  $\text{Fe}^{+2}$  oxidation with a stoichiometric ratio of 8:1 in molar units (Stumm and J.J., 1996). Additionally,  $\text{Fe}^{+2}$  can also be biologically removed by Fe-oxidizing microorganisms. Biological  $\text{Fe}^{+2}$  oxidation is stimulated at low dissolved oxygen concentrations and pH between 5.5 and 7.5 (Sharma et al., 2005).

Similarly  $\text{Mn}^{+2}$  can be chemically oxidized, precipitating on the filter bed predominantly as  $\text{MnO}_2$ , but possibly also as  $\text{Mn}_2\text{O}_3$ ,  $\text{Mn}_3\text{O}_4$  depending on the pH and the redox conditions (Crittenden et al., 2005). Biological removal may also occur by the action of Mn-oxidizing bacteria, especially at high dissolved  $\text{O}_2$  concentrations and at pH >7.5 (Mouchet, 1992).

$\text{NH}_4^+$  is biologically oxidized in two steps, i.e. oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , and conversion of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . Ammonium Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) attached to the filter material (Zhu et al., 2010) are believed to be responsible for the two nitrification steps. Both nitrification steps require oxygen as the electron acceptor at a ratio of 4.5 g  $\text{O}_2$ /g  $\text{NH}_4^+$ -N converted to  $\text{NO}_3^-$ . Complete conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  consumes also alkalinity by a stoichiometric ratio of 2:1 in molar units or 7 g  $\text{HCO}_3^-$ /g N- $\text{NH}_4^+$  (Metcalf & Eddy, 2004).

Simultaneous removal of  $\text{Fe}^{+2}$ ,  $\text{Mn}^{+2}$  and  $\text{NH}_4^+$  in rapid sand filters can be challenging, as these processes require different redox conditions and may affect each other (Crittenden et al., 2005). Redox conditions can stratify with depth, creating depth layers where each of the compounds is removed e.g.  $\text{Mn}^{+2}$  removal takes place after  $\text{NH}_4^+$  is completely oxidized to  $\text{NO}_3^-$  (Rittmann and Snoeyink, 1984; Mouchet, 1992; Vandenabeele et al., 1992).

## 1.6. Problems in rapid sand filters

Although rapid sand filters appear to be simple systems, they are actually complex environments with several physical, chemical and biological processes taking place at the same time. As a consequence, different problems in filter performance are likely to occur.

Investigation of poor performing filters starts with identification of the symptoms, followed by a preliminary diagnosis that is further investigated to ultimately suggest a potential remedial strategy (Lopato et al., 2012). Common symptoms in problematic filters are filter bed growth, short filter run and insufficient removal of particles and dissolved compounds from the water (Lopato et al., 2012). Filter bed growth and short filter run are typically caused by accumulation of particulate material and mineral deposits in the filter bed. Investigation of these cases normally includes examination of the physical state of the filter (Lopato et al., 2012).

Poor removal of particles and dissolved compounds can be more complicated to resolve, as it may be caused by several parameters affecting the physical state of the filter i.e. poor hydraulic design, flow channeling and filter clogging. Additionally, poor removal of dissolved compounds and especially of  $\text{NH}_4^+$  may be caused by low biological activity at the operation conditions. Indicatively, almost 20% of the waterworks in Denmark have experienced challenges to comply with the  $\text{NH}_4^+$  effluent guideline (Jupiter, 2013). In these cases, tentative remedial solutions are adopted that may or may not resolve the problem, making optimization expensive and time consuming.

## 1.7. Motivation of the project

From the above discussion, it is clear that rapid sand filters are not simple strainers and that performance is often compromised resulting in poor quality of the produced water. This project focused specifically on  $\text{NH}_4^+$  removal in rapid sand filters.

Nitrification has been well studied in soil and wastewater systems, but limited knowledge is available in oligotrophic environments such as biological filters for groundwater treatment. Most studies on nitrification in drinking water biofilters have focused on obtaining high  $\text{NH}_4^+$  removal rates and low effluent concentrations (Bray and Olanczuk-Neyman, 2001; Wolthoorn et al., 2004; Stembal et al., 2005; Lytle et al., 2007; Tekerlekopoulou et al., 2012) without examining nitrification dynamics in the filter. This approach creates a “black-box” view, where  $\text{NH}_4^+$  removal is averaged and assumed to be homogeneous in

the entire filter body. This may not necessarily be the case, and is one of the aspects we focused on in this work.

Furthermore, filters are microbially rich environments and several different populations have been identified on the filter material (Madoni et al., 2000; de Vet et al., 2009). It is not clear which of these microorganisms are contributing to nitrification and how the operational conditions affect their activity, ultimately affecting filter performance.

Lack of scientifically documented knowledge prevents optimization of filter design and operation. Up to date, filters are designed based on rules of thumb, with conservative safety factors to ensure that effluent water quality satisfies guideline requirements. Operational strategy is also based on experience (Lawler and Nason, 2006), making difficult the remediation of poor filter performance.

On the contrary, insight on nitrification biokinetics is a systematic approach to obtain knowledge on the relevant processes and parameters affecting nitrification in these systems. Detailed knowledge on nitrification kinetics can reveal potential process limitations as the basis for a targeted optimization strategy. Process understanding is not only needed to remediate poor performing filters, but is mainly needed to prevent their occurrence by suggesting a more efficient and robust filter design and management.

## 1.8. Objectives

Overall this PhD project addressed nitrification in rapid sand filters from a process understanding perspective. The general objective was to provide insight on  $\text{NH}_4^+$  removal biokinetics that is currently lacking in literature and that can be used for process optimization. The project was conducted in Denmark, and therefore focused on the needs and challenges of the Danish water supply system. However, as groundwater is worldwide used as a drinking water source, this project aimed not to be a case study, but to provide knowledge applicable in other similar systems. The specific aims addressed in this thesis were to:

- Develop an assay that can be used in site-specific filter investigations to detect and quantify nitrification activity
- Quantify nitrification activity in differently performing filters and identify potential stratification patterns
- Examine the presence and relation of mineral coating with stratification and nitrification activity and in a filter
- Analyze stratification in a filter based on the presence and the biokinetics of specific nitrifying microorganisms





## 2. Methods used to investigate nitrification biokinetics

### 2.1. Importance of scale in nitrification biokinetic investigations

Nitrification kinetics in rapid sand filters can be investigated at several scales. Selection of the appropriate scale is essential to obtain the correct information when addressing a specific objective. Each scale has different characteristics that can become advantages or disadvantages and make it suitable only for certain types of investigations.

Full-scale studies are the most accurate, as they truly reflect filter performance. Observations from full-scale filters include the effect of all parameters on nitrification, such as e.g. stratification with depth, hydrodynamic conditions and variations within filter run cycles. On the other hand, these studies are tedious and many precautions should be taken while working in a filter. These challenges include on site sterilization of all sampling equipment and wearing protective suits during the investigations, to avoid filter contamination that can pass on to the produced water. The main challenge in full-scale studies however, remains that the operation conditions cannot be modified if the filter is not excluded from production during the experimental time. This means that kinetic behavior can only be investigated at the operation conditions, limiting the range of information that can be gained.

Pilot-scale studies are suitable for a wide range of experimental objectives as they allow modification of the operation conditions, maintaining many characteristics of the full-scale such as depth distribution, attached biomass characteristics and hydrodynamic conditions. It is questionable nonetheless, if full-scale hydraulics and effect of backwashing are maintained in the pilot-scale, since geometry of the two systems is different. Moreover, experiments with pilot columns are time consuming and expensive, due to long start-up or stabilization times in addition to the extensive monitoring equipment required.

Bench-scale assays have the advantage of allowing complete control of the operation conditions that cannot be achieved in larger scales. The advantage over pilot-scale investigations is that the effect of one investigated parameter e.g. loading can be isolated from other parameters such as hydrodynamic conditions and depth stratification. Bench-scale experiments are usually simpler, faster and

cheaper than pilot-scale ones, and are suitable for site-specific studies since the obtained information does not reflect the behavior of the entire filter.

Molecular methods can identify the presence of specific microbial types and quantify their abundance. This information cannot be inferred by larger scales, although molecular methods do not provide any kinetic information.

This study used full-scale observations to capture nitrification biokinetics at in situ filter conditions, and bench-scale observations to extrapolate this kinetic behavior to a series of different loading conditions. The observations from both scales were related to the microbial abundances measured by molecular techniques.

## 2.2. Nitrification investigations in full-scale filters

### 2.2.1. $\text{NH}_4^+$ removal in full-scale filters

The first step to evaluate nitrification performance in a rapid sand filter is to measure  $\text{NH}_4^+$  influent and effluent concentrations. This is essential to assess the influent loading and the removal rate in the entire filter unit. The volumetric loading rate can be calculated by the influent  $\text{NH}_4^+$  concentration and the average flowrate to the filter, normalized by the packed bulk volume of the filter material. The volumetric removal rate can be calculated from the difference of influent and effluent  $\text{NH}_4^+$  concentration, multiplied with the average flowrate and normalized by the volume of sand in the filter.

Table 3 illustrates the influent and effluent  $\text{NH}_4^+$  concentrations, loading and removal rates in four full-scale filters. The investigated filters operated at different  $\text{NH}_4^+$  loading rates, ranging from 11.5 to 50.9 g  $\text{NH}_4^+/\text{m}^3/\text{d}$  and performed differently in terms of  $\text{NH}_4^+$  effluent concentrations and removal rates. Although influent and effluent water analysis allows an initial quantification of nitrification activity in a filter, this indirectly assumes that  $\text{NH}_4^+$  removal takes place equally in the entire filter depth. This may not be true in all filters, and a depth profile is needed to identify where nitrification takes place in a filter.

**Table 3.** Measured  $\text{NH}_4^+$  concentrations and calculated loading and removal rates in four full-scale filters in the greater Copenhagen area, Denmark

Islevbro <sup>a</sup>		Treatment Plant A <sup>b</sup>		Marbjerg <sup>a</sup>
		West line	East line	
Influent $\text{NH}_4^+\text{-N}$ (mg/L)	0.13	0.55	0.30	0.16
Effluent $\text{NH}_4^+\text{-N}$ (mg/L)	<0.01	0.05	0.10	<0.01
Volumetric $\text{NH}_4^+\text{-N}$ loading rate ( $\text{g/m}^3/\text{d}$ )	17.8	50.9	18.5	11.5
Volumetric $\text{NH}_4^+\text{-N}$ removal rate ( $\text{g/m}^3/\text{d}$ )	17.8	46.3	12.3	11.5

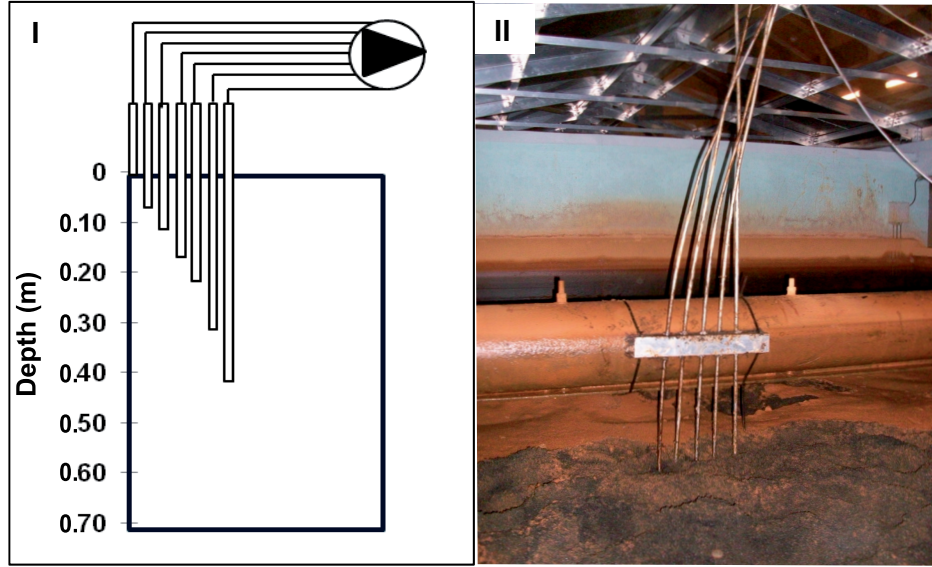
<sup>a</sup>Operated by Hofo A/S, <sup>b</sup>The name is not disclosed as this treatment plant preferred to be anonymous

### 2.2.2. $\text{NH}_4^+$ depth profiling

$\text{NH}_4^+$  depth profiling in a filter can reveal the actively nitrifying regions and can be used to extract a kinetic expression that describes the observed  $\text{NH}_4^+$  concentration decrease with depth.

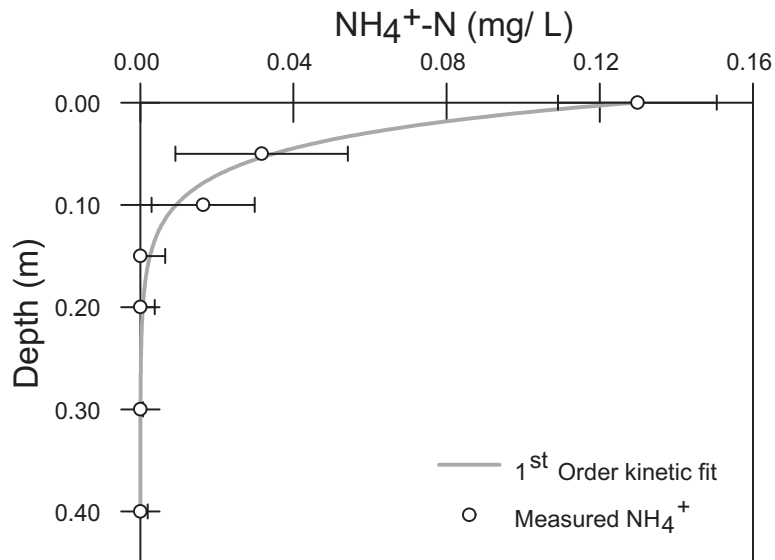
Depth profiles can be obtained by inserting pipes in the filter, with the one end at a selected filter depth and the other end connected to a pump. The simplest configuration consists of one individual pipe sampling water from one specific depth each time. The pipe may be inserted in the filter vertically or with an angle to avoid disturbance of the flow path above the sampling point (Lopato et al., 2013). Alternatively, several depths may be sampled simultaneously if pipes of different lengths are combined in a multi-level sampler.

A multilevel sampler was used to sample water from the Islevbro filter presented in Table 3. The sampler consisted of 5 stainless steel pipes, fixed in the middle of the filter. The pipes were connected to a multichannel pump and sampled water the 0.05-0.40 m depth region (Figure 4). The pipes were installed with 5 cm distance between each other to avoid overlapping of the sampling water volumes around the pipe ends. We selected this sampling configuration, where the pipes are permanently installed in the filter, to observe if and how the depth  $\text{NH}_4^+$  profile changes within a filter run cycle and in different filter run cycles. The filter was sampled 6 times during 2 filter run cycles, each lasting 14-15 days and the average depth  $\text{NH}_4^+$  profile is illustrated in Figure 5.



**Figure 4.** (I) Schematic illustration and (II) picture of the sampling pipes installed at the Islevbro full-scale filter.

$\text{NH}_4^+$  concentrations at the investigated depths did not change significantly from day to day, indicating that  $\text{NH}_4^+$  removal was not affected by time after the last backwashing (Figure 5). The measured  $\text{NH}_4^+$  concentrations were fitted to a 1<sup>st</sup> order decrease model predicting the  $\text{NH}_4^+$  concentration at a specific filter depth (Tatari et al., I).



**Figure 5.** Average  $\text{NH}_4^+$  depth profile observed at the Islevbro full-scale filter. Error bars show the standard deviation of measurements. Observed concentrations were fitted into the 1<sup>st</sup> order decrease expression  $S_{\text{NH}_4^+} = 0.13 \exp^{-0.26d}$  (Tatari et al., I)

$\text{NH}_4^+$  was not detected (LOD  $\text{NH}_4^+\text{-N}$  0.01 mg/L) in any sampling campaign at depths below 0.20 m. Removal was concentrated in the top 0.20 m of the filter, while deeper layers did not appear to be active for nitrification as they did not receive a detectable  $\text{NH}_4^+$  loading. It cannot be inferred from the depth profile if filter layers deeper than 0.20 m have an inherent nitrification activity that can be activated if  $\text{NH}_4^+$  penetrates deeper in the filter. This activity can only be detected in offline bench-scale investigations, where the filter material is exposed to higher  $\text{NH}_4^+$  loading rates.

## 2.3. Nitrification biokinetics in bench-scale assays

### 2.3.1. Types of bench-scale assays

Bench-scale assays have the advantage of allowing modification of operation conditions, which cannot be done in a full-scale filter. In the example presented in Figure 5, a bench-scale assay can be used to quantify the nitrification activity of filter layers below 0.20 m that cannot be investigated in the full-scale filter.

Respirometry is a common assay type used to quantify nitrification activity via oxygen depletion measurements (Chandran and Smets, 2001). However, respirometry normally requires biomass detachment prior to the assay. This method has been used to quantify nitrification activity in rapid sand filters (Madoni et al., 2001), although it cannot capture biofilm kinetics since the biomass has been detached from the filter material. An alternative is the batch assay suggested by Kihn et al., 2000 and used by De Vet et al., 2009, where the filter material is stirred in a medium spiked with an initial  $\text{NH}_4^+$  pulse. The decrease of the  $\text{NH}_4^+$  concentration is followed with time to calculate the specific activity per filter material volume. The disadvantage of this method is that mechanical stirring can also cause abrasion and detachment of cells from the filter material. Instead, the use of a packed column assay can ensure that biofilm integrity is maintained and that true biofilm kinetics is reflected. Within this work, we developed an assay that allowed control of the  $\text{NH}_4^+$  loading conditions, and included potential internal and external mass transfer limitations.

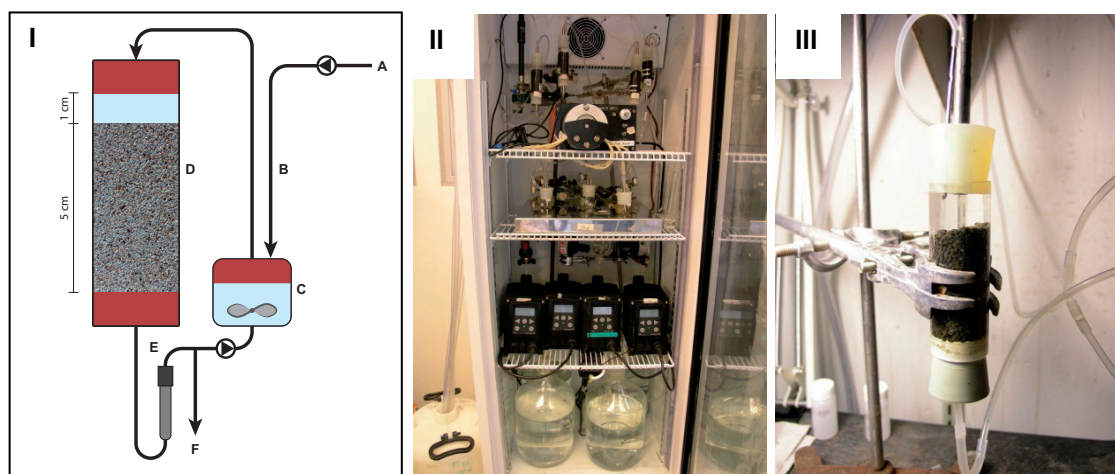
We used the bench-scale assay in investigations that quantified nitrification activity at different filter depths, estimated growth kinetics at steady-state and dynamic loading conditions, evaluated the use of specific nitrification inhibitory compounds and examined the effect of mineral coating presence on nitrification activity.

Furthermore, this assay can be suitable for studies investigating e.g. potential nutrient limitations in nitrification, effect of iron or manganese on nitrification and co-metabolic degradation of micropollutants. Although this assay was developed and validated for activity investigations in rapid sand filters, its use can be extended to other growth media e.g. anthracite, expanded clays and activated carbon.

### 2.3.2. Principles & use of the developed bench-scale assay

The developed assay aimed to enable investigation of site-specific nitrification kinetic behavior at different loading conditions that cannot be created in the filter. These investigations can quantify the maximum and the inherent nitrification activity in filter layers that do not receive a detectable  $\text{NH}_4^+$  loading in the filter.

For these investigations, filter material was collected from the full-scale. Once in the lab, the sand was packed in small-scale columns (inner diameter 2.6 cm, bed height 5 cm). Each set-up consisted of the column and a recirculation stream and was continuously operated at 10°C to match the average water temperature at the waterworks (Figure 6).



**Figure 6.** (I) Sketch of the experimental set-up, where: A: Influent reservoir, B: Influent stream, C: Mixing vessel, D: Packed column with 1 cm overlay water on top of sand bed, E: Recirculation stream and on-line dissolved oxygen monitoring, F: Effluent sampling point. (Tatari et al., I). (II) Picture of experimental set-up. (III) Detail of one column used in the bench-scale assay

Loading and external mass transfer were controlled in the bench-scale assay. Loading is determined by the influent concentration and the flowrate, and can be modified by adjusting one of the two parameters. We selected to adjust the flow because an increase in influent concentration may cause oxygen depletion due to no aeration in the system, while a decrease may result in analytical challenges to quantify  $\text{NH}_4^+$ .  $\text{NH}_4^+$ -N influent concentration was 1 mg/L, constant at all loading levels.

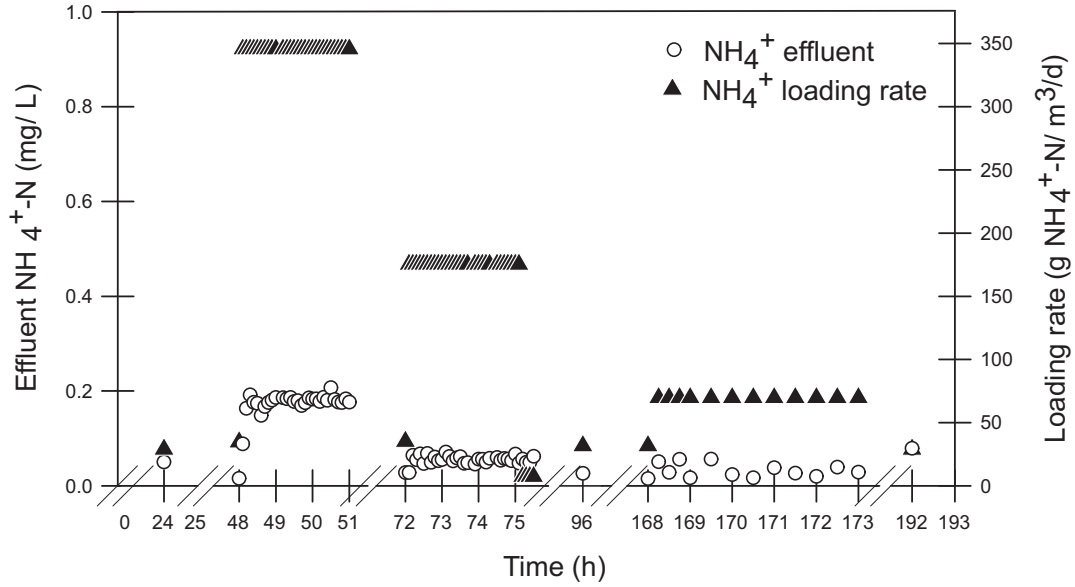
External mass transfer conditions were maintained in the assay to isolate and observe the biokinetic responses at different loading levels. External mass transfer is controlled by the boundary layer thickness, which is a function of the hydraulic loading rate (superficial velocity). Full-scale mass transfer conditions were imposed in the bench-scale by matching the hydraulic loading rate of the filter.

Nitrification biokinetic behavior was investigated by monitoring the effluent  $\text{NH}_4^+$  concentration response at a series of steady-state and dynamic loading conditions. To illustrate the responses at these different loading conditions, the experiments with filter material from the top 0-0.10 m of the Islevbro filter are presented in this section. Variation of effluent  $\text{NH}_4^+$  concentration at varying loading conditions between 35 g  $\text{NH}_4^+$ -N/m<sup>3</sup>/d (reference loading) and 350 g  $\text{NH}_4^+$ -N/m<sup>3</sup>/d is shown in Figure 7.

$\text{NH}_4^+$  effluent right after start-up (1 day after) reflects the nitrification activity of the filter material in the full-scale, as no substantial growth of nitrifying microorganisms can occur within 1 day of operation. Column performance 1 day after-start-up is used in section 3.1 to evaluate the distribution of nitrification activity with depth in a filter. In the experiment illustrated in Figure 7, we selected the reference loading in the range of full-scale loading conditions for this filter depth region.

Response to short-term loading variations indicates how the column performance is affected by the loading level. These investigations can be used to identify the maximum nitrification rate of a filter material sample. Short-term loading up-shifts caused an immediate effect on the effluent concentration that stabilized to a new temporary steady-state for each loading condition (Figure 7). However, when loading was decreased back to reference, antecedent performance was re-established suggesting that transient loading up-shifts do not affect the nitrifying biomass.



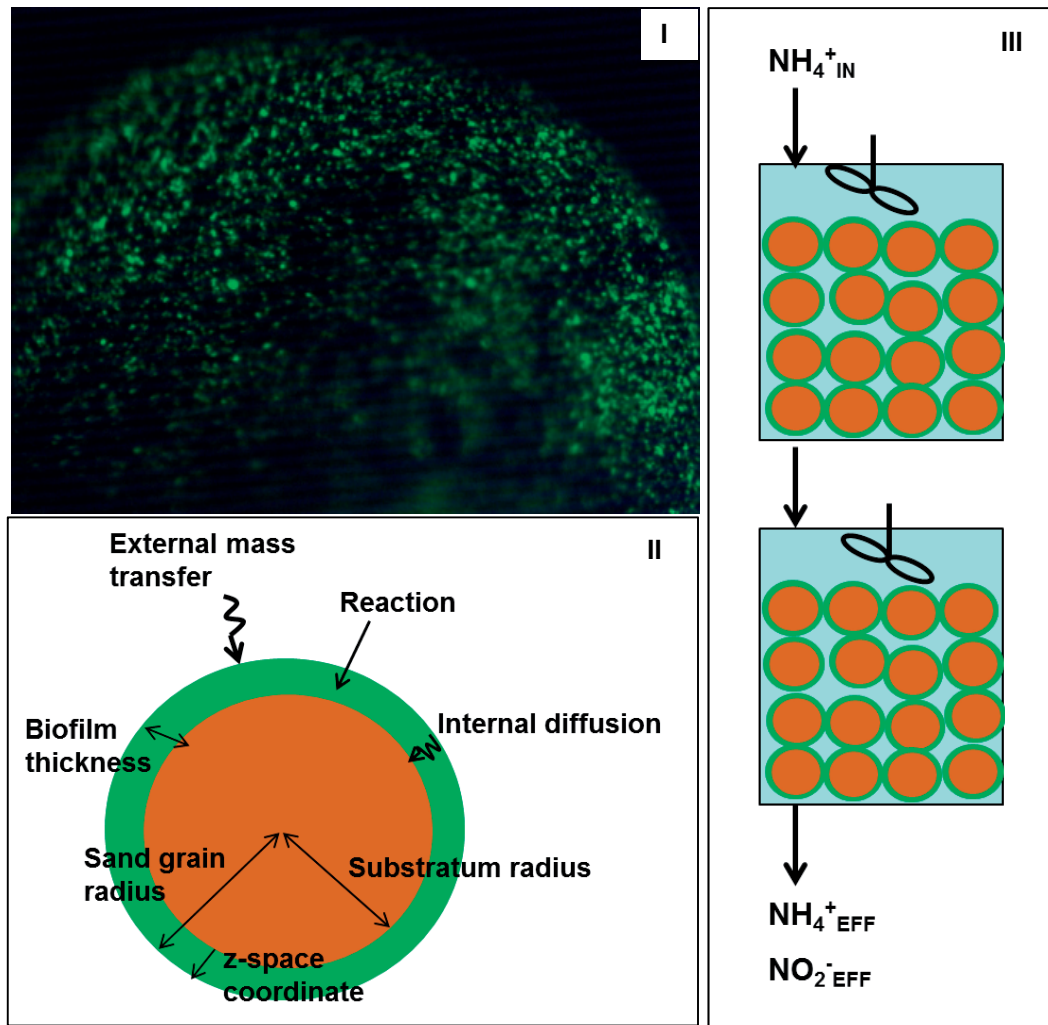


**Figure 7.** Applied loading rates and response in  $\text{NH}_4^+$  effluent concentration in the bench-scale assay (adapted from Tatari et al., I)

## 2.4. Mathematical modeling of nitrification kinetics in the bench-scale assay

Removal observed in the lab-scale columns can confirm the presence of nitrification activity in selected filter sections and can quantify the effect of loading increase within the investigated range. However, a mathematical model is needed to estimate the kinetic parameters that describe the effluent behavior at the applied loading conditions, and to further interpret the observed trends. A kinetic expression can relate column performance with the number and growth rate of nitrifying microorganisms, potentially suggesting process limitations. Moreover, kinetics can predict performance at a wider range of conditions, allowing deeper insight on nitrification in these systems.

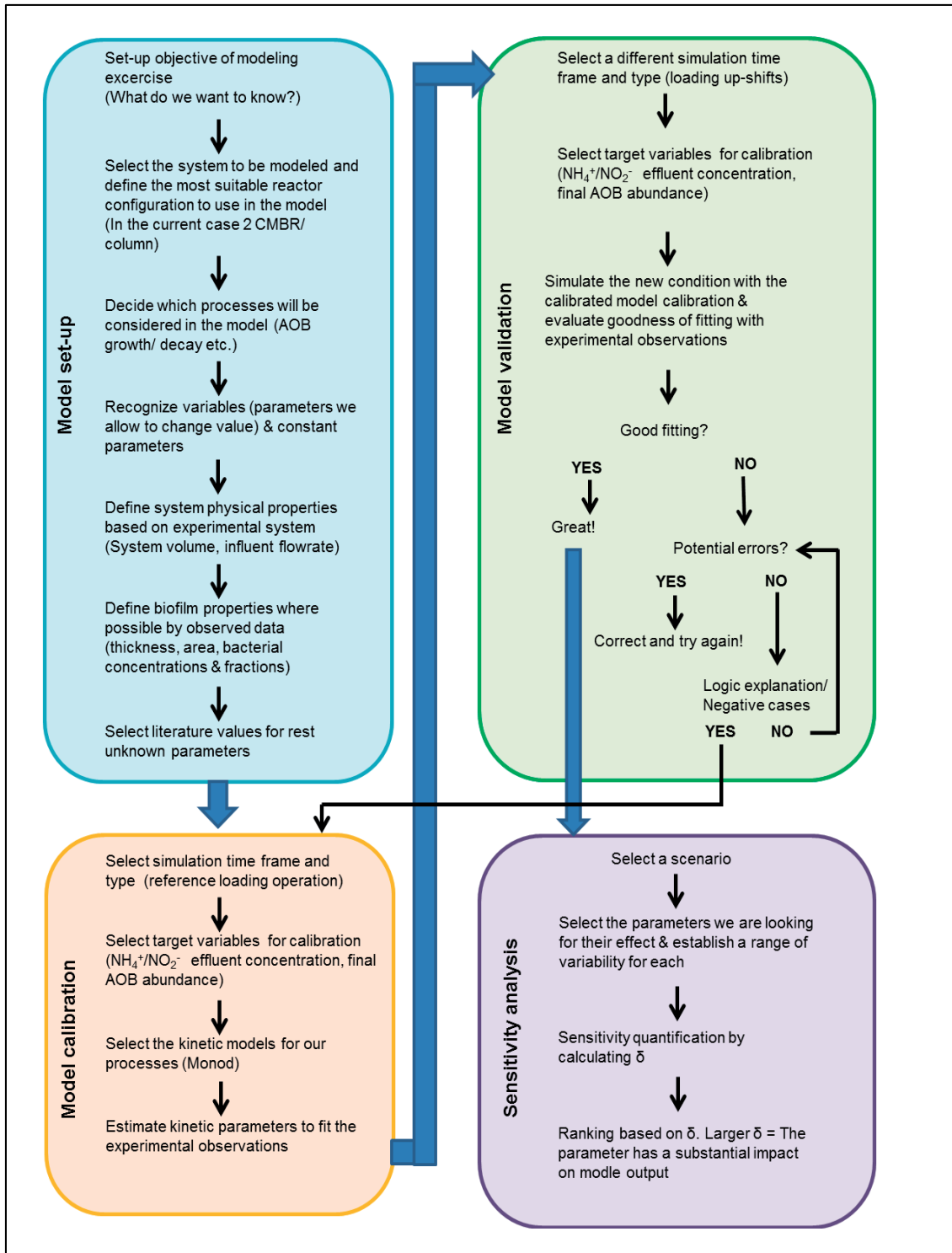
Based on microscopic observations (Figure 8 I), we observed that microorganisms attached to the sand grains in a rapid sand filter were uniformly spread on the outer sand grain periphery. This allowed us to assume a continuous and homogenous biofilm on the sand grain surface as illustrated in Figure 8 II. The biokinetic behavior was described by a 1-D model that considered reaction and mass transfer at the biofilm level to calculate the effluent  $\text{NH}_4^+$  and  $\text{NO}_2^-$  concentration at the lab-scale column level.



**Figure 8.** (I) View of the external surface of a sand grain by confocal laser scanning microscopy. Green areas are stained microbial clusters (Courtesy of Arda Gülay). (II) Conceptual model on the sand grain scale. (III) Conceptual model on the lab-scale column scale

The biofilm surface area was expressed as function of the distance from the sand substratum by the surface area of a spherical shell at each location on the z-axis. The total biofilm area was calculated by multiplying the surface area of each sand grain with the number of grains in the column. The physical system was modeled as 2 mixed compartments in series (Figure 8 III) to fit the hydrodynamic conditions characterized by salt tracer tests in the column (Tatari et al., I). Nitrification was modeled in two steps, oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  (nitritation) by AOB and  $\text{NO}_2^-$  oxidation to  $\text{NO}_3^-$  by NOB (nitrification). The effluent concentration was calculated by the model based on mass balances.

The model was built and calibrated following the series of steps depicted in Figure 9. Model calibration aimed to identify the best fit kinetic parameters describing the observed behavior. Decay coefficient  $b$  ( $d^{-1}$ ), maximum growth rate  $\mu_{max}$  ( $d^{-1}$ ) and half saturation concentration for  $NH_4^+$  (mg/L) were estimated for AOB from model calibration. Parameter estimation targeted to fit the effluent  $NH_4^+$  and  $NO_2^-$  concentrations, and the final AOB abundance quantified the end of the column experiments (Tatari et al., **II**).



**Figure 9.** Flowchart of steps followed during the modeling investigations. Adapted from Boltz et al., 2011 and Boltz et al., 2013



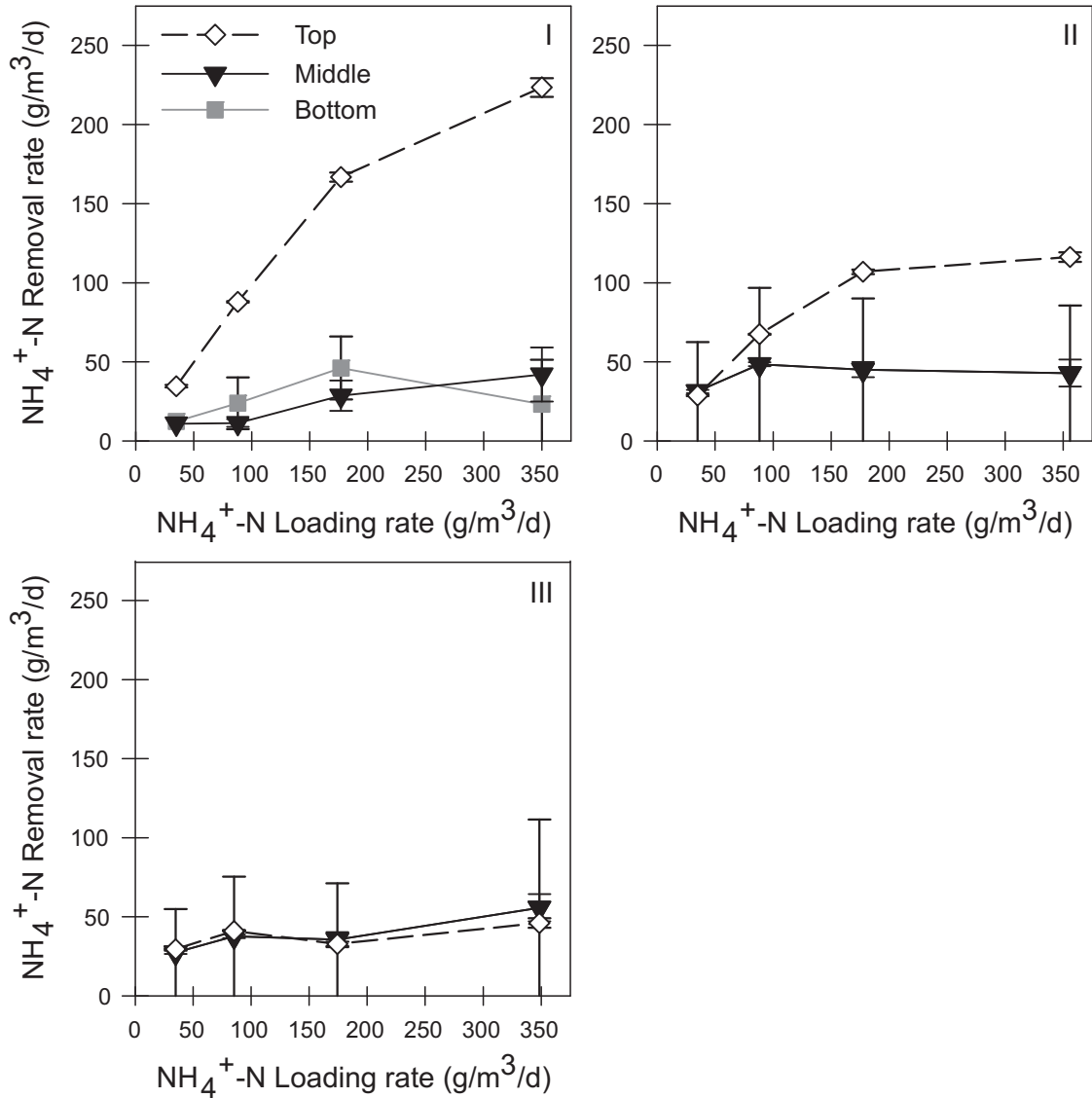
### 3. Stratification of nitrification activity with depth

#### 3.1. Distribution of nitrification activity with depth in different filters

Depth profiling can indicate where  $\text{NH}_4^+$  is removed in a filter, but cannot provide information on how the nitrification activity is distributed with depth. Investigation of nitrification activity at different filter depths can indicate the presence of potential stratification patterns that can affect filter performance.

The previously presented filter at Islevbro waterworks (Table 3, Figure 5) and one after-filter at the West and East line of Treatment Plant A (Table 3) were investigated for the distribution of nitrification activity with depth. The filters were core-sampled to collect filter material from three depth regions of the filter at Islevbro: 0-0.10 m (top), 0.20-0.30 m (middle) and 0.35m-0.50 m (bottom). Filter material was collected from two depth layers: 0-0.10 m (top) and 0.20-0.30 m (middle) of the after-filters at Treatment Plant A. Nitrification activity of each depth layer was quantified in the developed bench-scale assay at loading conditions between 35 and 350 g  $\text{NH}_4^+$ -N/m<sup>3</sup>/d.

$\text{NH}_4^+$  removal of the top Islevbro filter layer was higher than the removal of middle and bottom layers at all loading levels, suggesting stratification of nitrification activity with depth (Figure 10 I). Removal observed in the filter material from the Treatment Plant A West line suggested that nitrification activity was stratified with depth, although the difference between top and middle was less pronounced than in the Islevbro filter (Figure 10 II). Nitrification activity did not appear to be stratified in the Treatment Plant A East filter, since removal of the top and middle layers was similar.



**Figure 10.** (I) Removal at each loading condition in filter material collected from different depth layers of the Islevbro after-filter (adapted from Tatari et al., II), (II) Treatment Plant A West line after-filter and (III) Treatment Plant A East line after-filter

To interpret the observed stratification patterns, we determined the abundance of AOB in the initial filter material samples as reported in Table 4. AOB abundance was consistent with the nitrification activity stratification patterns in all filters. More AOB cells were detected in the top layer of the Islevbro and Treatment Plant A West filters compared with the deeper layers, whereas similar cell numbers were quantified at both depths of the Treatment Plant East filter (Table 4). This suggested that stratification of AOB abundance was a plausible explanation for the distribution of activity with depth.

However, it remains peculiar, how the roughly 1-2 orders of magnitude higher AOB cell numbers found in the Treatment Plant A filters yielded lower removal rates at all loading levels compared with the Islevbro filter.

**Table 4.** AOB abundance per filter material packed bulk volume in the different depth layers of the three examined filters. Values are reported with the standard deviation of triplicate measurements. Please note that the values are reported in different orders of magnitude

Depth layer	Islevbro $\times 10^{12}$ cells/m <sup>3</sup>	Treatment Plant A West $\times 10^{14}$ cells/m <sup>3</sup>	Treatment Plant A East $\times 10^{14}$ cells/m <sup>3</sup>
Top	31 $\pm$ 1.2	13 $\pm$ 2.2	1.6 $\pm$ 0.4
Middle	2.3 $\pm$ 0.4	2.1 $\pm$ 0.1	1.9 $\pm$ 0.3
Bottom	6.2 $\pm$ 3.7	-	-

### 3.2. Link of nitrification activity stratification with filter performance

The three examined filters were operated at different  $\text{NH}_4^+$  loadings and were performing differently as indicated in Table 3. The Islevbro filter has constantly delivered an effluent  $\text{NH}_4^+$  concentration below 0.01 mg/L. The depth stratification profile of activity and AOB cell abundance is consistent with the  $\text{NH}_4^+$  depth profile in the filter (Figure 5), showing a steep substrate concentration decrease with depth. The depth profile revealed that nitrification was taking place mainly in the top layer, and no  $\text{NH}_4^+$  was detected below 0.20 m. However, nitrification activity was detected in the middle and bottom layers when exposed to higher loadings in the lab-scale columns. This inherent nitrification activity of the deeper filter layers may function as an excess capacity in case  $\text{NH}_4^+$  penetrates deeper, due to influent water quality changes or peak hydraulic loadings. It is questionable nevertheless, if this filter is oversized for  $\text{NH}_4^+$  removal, as the 0.50 m below the top layer do not contribute to nitrification in the full-scale.

The two Treatment Plant A filter lines were operated at different loading conditions and performed differently in terms of effluent  $\text{NH}_4^+$  concentration (Table 3). The Treatment Plant A West filter delivered an  $\text{NH}_4^+$ -N effluent concentration of 0.05 mg/L, whereas the Treatment Plant A East line filter



effluent was 0.1 mg/L, exceeding the guideline limit during the investigated period. Overall, stratification of nitrification activity appeared to be related with filter performance in the three examined cases, with the well performing filter being strongly stratified, the nearly well performing filter being stratified and the poor performing filter showing no stratification of activity and AOB abundance with depth.

### 3.3. Mineral coating properties and effect on stratification

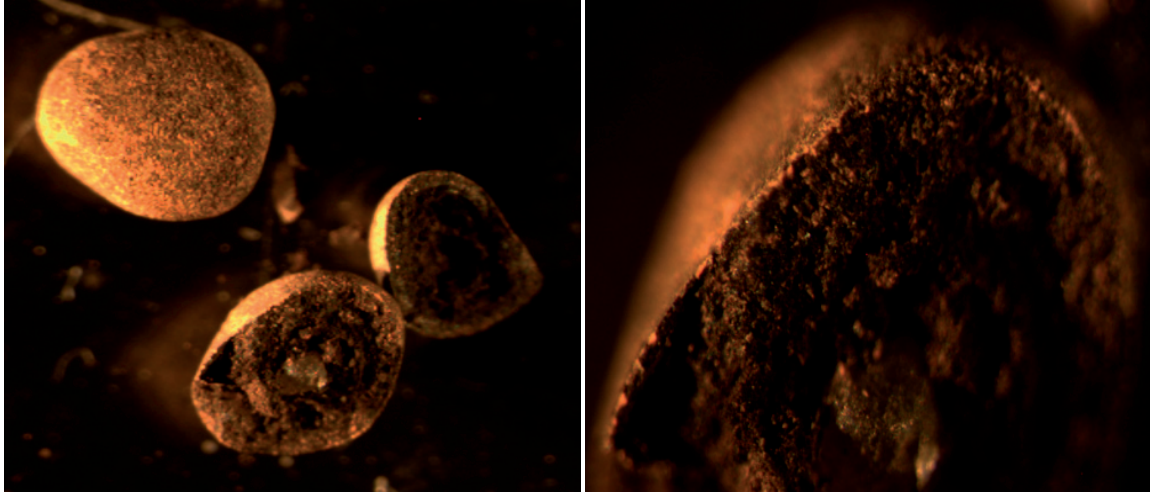
Strong stratification of nitrification activity and cell numbers is not expected in rapid sand filters, because backwashing partially lifts and mixes the filter material (AWWA, 1999). However, backwashing strategy and frequency may dictate stratification in filters with wide particle size distributions, settling the smaller sand grains in the top. The strong stratification pattern observed in the Islevbro filter was investigated with regards to the filter material physical properties. This filter has been in operation for nearly 30 years since the last filter material replacement, and is backwashed every 14-15 days with air scouring followed by high water up-flow.

The average sand grain size was estimated and revealed that sand grains were larger in the top filter layer (Table 5). The filter material packed bulk density indicated that the large sand grains found in the top of the filter were lighter and thus settled there after backwashing (Table 5).

**Table 5.** Physical characteristics of the coated sand in the three depth layers of the Islevbro filter

Depth layer	Average sand grain diameter (mm)	Drained-wet packed bulk density $\times 10^3$ (Kg/m <sup>3</sup> )	Mass coating extracted per dry sand mass (Kg/Kg)
Top	1.4	1.1	0.76
Middle	0.8	1.6	0.13
Bottom	1.1	1.7	0.06

On the external surface of the sand grains, we observed the presence of a brittle brown mineral coating. Microscopic view of sliced sand grains illustrated how the mineral coating surrounded the sand cores (Figure 11).



**Figure 11.** Microscopic view of sliced sand grains from the top layer of the Islevbro filter with the mineral coating surrounding the sand core (Courtesy of Arda Gülay)

The sand was treated with strong acids to remove the mineral coating and estimate its mass per filter material packed bulk volume (Table 5). Mineral coating was dominant in the top filter layer but was also found to a limited extent in deeper filter layers. Presence of mineral coating certainly affects the physical properties of the filter material (Hu et al., 2004; Pedersen et al., 2005; Sharma et al., 2005; Sahabi et al., 2009). Analyzing the average grain size and the density of the naked sand, we observed that mineral coating increased the average particle size especially of the top layer particles, while decreased the density (Table 6).

The presence of mineral coating indicated that periodic backwashing did not remove all mineral deposits from the filter bed, which instead accumulated with time and formed a permanent coating. Mineral coating is generally not desired in rapid sand filters, because it increases the filter material volume causing growth of the filter bed. In this part of the work, we observed that the presence of mineral coating and its effect on the filter material physical properties can dictate the stratification pattern in a filter.

Although several studies have reported the presence of mineral coating in mature biofilters for drinking water treatment (Vandenabeele et al., 1992; Hu et al., 2004; Schwartz et al., 2009; White et al., 2012), no investigations have quantified its effect on the physical properties of the filter material and filter stratification.

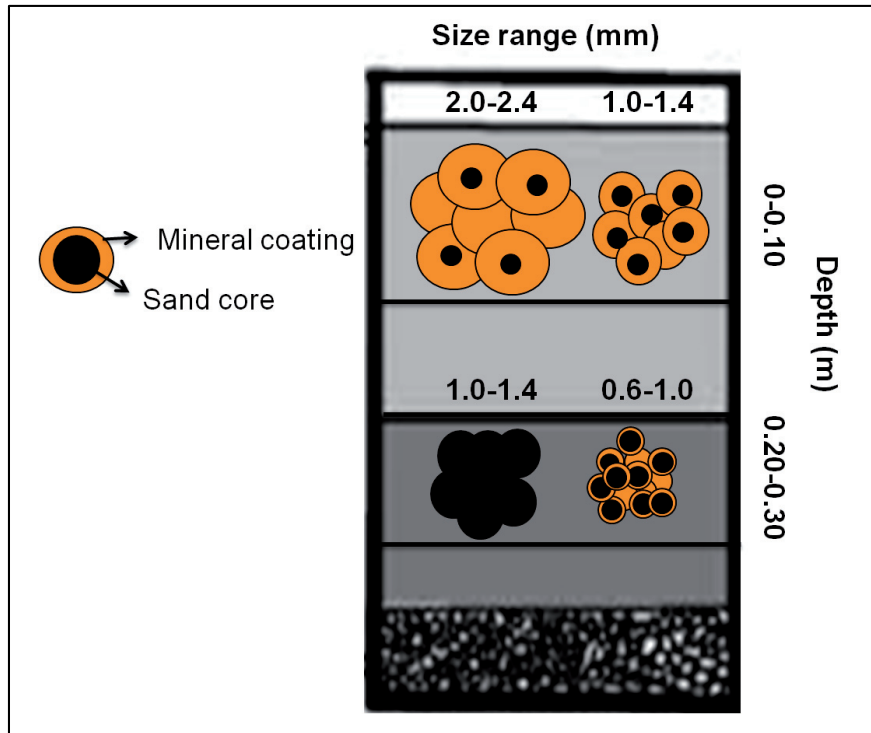
**Table 6.** Physical characteristics of the naked quartz sand after the coating was removed at the three depth layers of the Islevbro filter

<b>Depth layer</b>	<b>Average naked sand grain diameter (mm)</b>	<b>Drained-wet packed bulk density <math>\times 10^3</math> (Kg/m<sup>3</sup>)</b>
<b>Top</b>	0.7	2.6
<b>Middle</b>	0.8	2.6
<b>Bottom</b>	1.1	2.6

### 3.4. Correlation of nitrification activity with mineral coating presence and surface area

To further interpret the depth stratification profile and the implications of mineral coating presence, we investigated how coating and physical properties relate with nitrification activity. We collected filter material from the top and middle layer of the Islevbro after-filter, and segregated each depth layer according to sand grain size to obtain 4 filter material samples with different mineral coating properties as illustrated in Figure 12.

As observed previously, more mineral coating was present in the top filter layer, but coating extent varied also with sand grain size as indicated in Table 7. Density observations showed a decrease with more mineral coating present (Table 7). This density decrease, suggests that mineral coating is a porous structure, as confirmed also by the internal pore volume measurements (Gülay et al., IV). Larger internal pore volume (intra-grain void volume) per filter material packed bulk volume was estimated in the samples with more mineral coating (Table 7).



**Figure 12.** Sketch of the sampling strategy and the four filter material sub-samples obtained for the experimental investigations

Nitrification activity of the four filter material samples was quantified in the bench-scale assay at two loading conditions. The average removal at the two loading levels positively correlated with the presence of more mineral coating per packed bulk filter material volume (Figure 13 I).

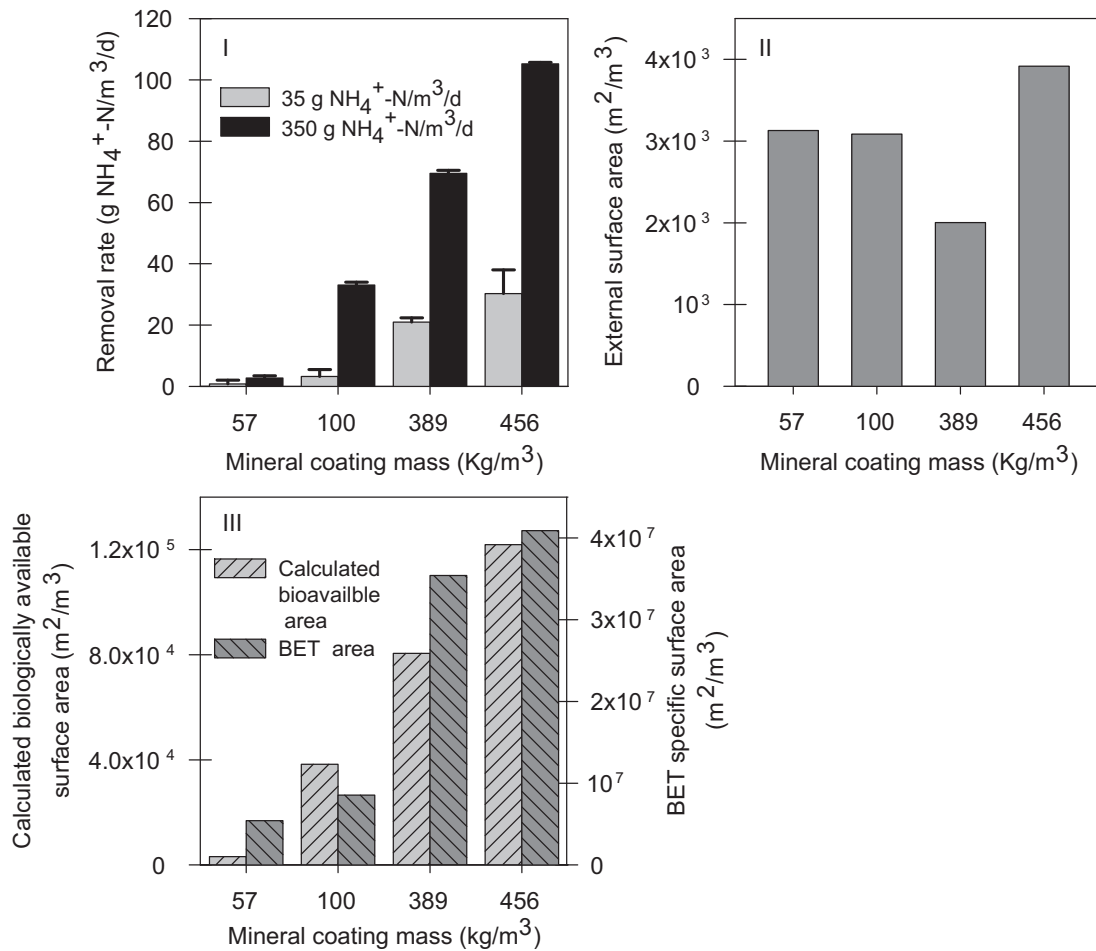
**Table 7.** Physical properties of the four filter material samples (adapted from Gülay et al., IV)

Depth layer	Size interval (mm)	Mass coating extracted (Kg/Kg) <sup>a</sup>	Mass coating extracted (Kg/m <sup>3</sup> ) <sup>b</sup>	Drained-wet packed bulk density ×10 <sup>3</sup> (Kg/m <sup>3</sup> ) <sup>b</sup>	Internal pore volume (m <sup>3</sup> /m <sup>3</sup> ) <sup>b</sup>
<b>Top</b>	2.0-2.4	0.78	456	1.1	0.57
	1.4-1.0	0.55	389	1.3	0.57
<b>Middle</b>	1.4-1.0	0.04	57	1.6	0.24
	1.0-0.7	0.07	100	1.7	0.26

<sup>a</sup>Expressed per filter material mass, <sup>b</sup>Expressed per packed bulk filter material volume

To interpret the activity trend, we calculated the external surface area of spherical sand grains and examined the correlation with the observed removal. The external surface area in the four samples did not follow the activity trend with the amount of mineral coating present (Figure 13 II). Instead, it appeared that nitrification activity correlated with the internal surface area as measured by the BET isotherm and as calculated in Gülay et al., IV (Figure 13 III).

Overall, mineral coating is related with nitrification activity in two ways: by changing the filter material density and imposing a stratified distribution of sand grains with depth, and by a creating a greater internal surface area that correlates with higher nitrification activity.



**Figure 13.** (I) NH<sub>4</sub><sup>+</sup> removal observed at the two loadings as function of the mineral coating mass per packed bulk filter material volume (Gülay et al., IV). (II) External surface area calculated as function of the mineral coating mass per packed bulk filter material volume. (III) Calculated bioavailable area equivalent to accommodate the observed NH<sub>4</sub><sup>+</sup> removal and experimentally determined BET surface area as function of the mineral coating per filter material volume (Gülay et al., IV)

## 4. Site-specific nitrification kinetics and physiology in rapid sand-filters

### 4.1. Active ammonium oxidizing species

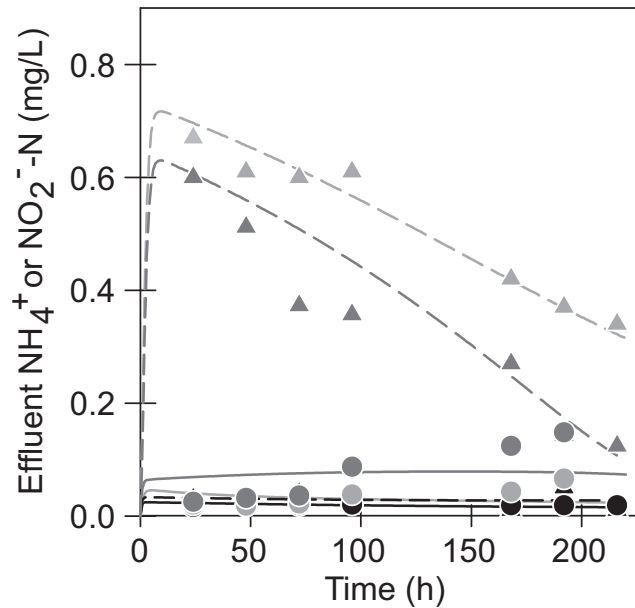
$\text{NH}_4^+$  oxidizing activity is typically attributed to AOB, who have until recently been considered the only microorganisms involved in this nitrification step. However, Ammonium Oxidizing Archaea (AOA) have been identified in several environmental systems, such as soil and marine sediments (Martens-Habbena and Stahl, 2011), and drinking water trickling filters (de Vet et al., 2009). Although several studies have investigated  $\text{NH}_4^+$  oxidation by AOA in pure or enriched cultures (Martens-Habbena et al., 2009; Jung et al., 2011; Santoro and Casciotti, 2011; Kim et al., 2012; Lehtovirta-Morley et al., 2013), their contribution to nitrification in complex environmental systems is not clear yet.

AOA half saturation concentration has been estimated in pure and enriched cultures within 0.002-0.42 mg/L  $\text{NH}_4^+$  (Martens-Habbena et al., 2009; Jung et al., 2011), which is lower than the typical AOB reported range of 0.057-1.75 mg/L  $\text{NH}_4^+$  (Prosser, 1989). High AOA affinity suggests that their activity is stimulated at low  $\text{NH}_4^+$  concentrations (Martens-Habbena and Stahl, 2011; Kim et al., 2012). Oligotrophic conditions that characterize drinking water biofilters and  $\text{NH}_4^+$  concentrations below detection limit in deep filter layers may therefore promote AOA nitrification.

Growth of AOB and AOA was investigated in bench-scale column experiments with filter material from the top, middle and bottom layer of the Islevbro after-filter to recognize the dominant active microbial types (Tatari et al., II). After 9 days of operation at reference loading (35 g  $\text{NH}_4^+\text{-N}/\text{m}^3/\text{d}$ ), the decreasing effluent  $\text{NH}_4^+$  concentration in the middle and bottom layers suggested growth of nitrifying microorganisms (Figure 14). AOB cell abundances measured before and after the column experiments indicated growth of AOB in the middle and bottom layers, confirming their activity in the columns (Tatari et al., II). AOA cell numbers instead, did not change during the experiment i.e. AOA activity could not be confirmed (Tatari et al., II).

A potential explanation is that  $\text{NH}_4^+$  concentrations in the middle and bottom columns (Figure 14) were too high to stimulate AOA activity and growth, although AOA growth was also not detected in the top column where  $\text{NH}_4^+$  concentration was considerably lower.

However, AOA were present in all depth layers at abundances in the same order of magnitude with AOB, but with no distinct stratification profile (Tatari et al., **II**). Comparable AOA and AOB abundances indicate that potentially both microbial types are active in the filter, although our observations could not confirm this hypothesis.



**Figure 14.** Observed and model predicted  $\text{NH}_4^+$  and  $\text{NO}_2^-$  effluent concentration in the columns at reference loading. Observed  $\text{NH}_4^+$ :  $\blacktriangle$  Top  $\triangle$  Middle,  $\blacktriangle$  Bottom. Observed  $\text{NO}_2^-$ :  $\bullet$  Top,  $\bullet$  Middle,  $\bullet$  Bottom. Model predicted  $\text{NH}_4^+$ : --- Top, --- Middle, --- Bottom. Model predicted  $\text{NO}_2^-$ : — Top, — Middle, — Bottom (Tatari et al., **II**)

## 4.2. Nitrification kinetics at different loading conditions

Performance of each depth layer in the above column experiments was kinetically described by a mathematical model. The model was calibrated at reference loading to estimate the best fit growth and decay parameters. Predicted effluent  $\text{NH}_4^+$  and  $\text{NO}_2^-$  concentrations are shown in Figure 14. Estimated half saturation concentrations ranged from 0.15 to 0.016 mg/L  $\text{NH}_4^+$ -N, resulting in 1<sup>st</sup> order kinetic behavior in the top layer and zero order in the middle and bottom layers (Tatari et al., **II**). Maximum growth rates were 0.249, 0.457 and 0.204 d<sup>-1</sup> in the top, middle and bottom layers respectively (Tatari et al., **II**).

However, when exposed to higher loading levels (88-350 g  $\text{NH}_4^+\text{-N}$  / $\text{m}^3/\text{d}$ ), the top and middle layer indicated an 3.4 and 2 fold up-shift of the maximum growth rate, whereas up-shift capacity was not observed in the bottom filter layer (Tatari et al., II). This behavior suggested an up-shift in metabolic activity with loading increase that has not been reported previously in literature.

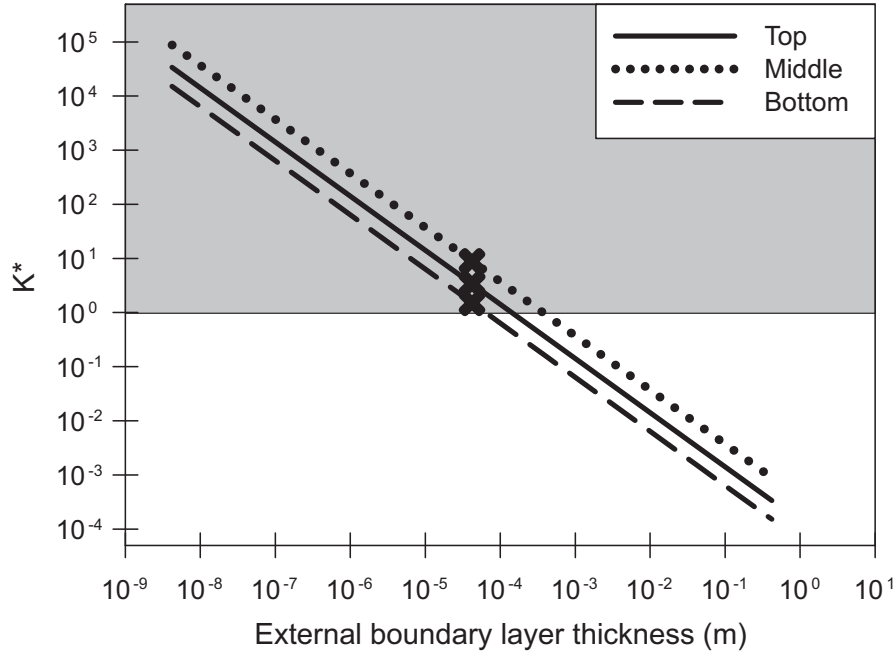
Only few studies have described nitrification kinetics in drinking water biofilters (Uhl and Gimbel, 2000; Queinnec et al., 2006). In their work, Uhl and Gimbel used literature kinetics for pure *Nitrosomonas* cultures to predict the performance of a rapid sand filter. Queinnec and colleagues estimated a 0.4 mg/L  $\text{NH}_4^+\text{-N}$  half saturation concentration and used a fixed 0.26  $\text{d}^{-1}$  (after temperature conversion to 10°C) maximum growth rate for AOB. Although nitrification has been widely investigated in wastewater systems, behavior at high nutrients' concentrations may not be applicable at the oligotrophic conditions in drinking water filters. This means that very limited kinetic information is available for drinking water biofilters, highlighting the significance of the present work.

### 4.3. External mass transfer limitations in rapid sand filters

Boundary layer retardation was examined via the dimensionless number  $K^*$  that expresses the ratio of internal biofilm processes i.e. reaction and diffusion with external mass transfer limitations (Rittmann and McCarty, 2001).  $K^*>1$  indicates that nitrification rate is determined by biofilm processes, whereas  $K^*<1$  indicates significant external mass transfer limitations. We explored  $K^*$  in each depth layer for hypothetical external boundary layer thicknesses ranging within  $4.1\times 10^{-9}$ -  $4.1\times 10^{-1}$  m (Figure 15). The calculated external mass transfer boundary layer thickness in the column experiments (and in the full-scale filter) was  $4.2\times 10^{-5}$  m (42  $\mu\text{m}$ ) (Tatari et al., I) and the corresponding  $K^*$  is illustrated in Figure 15 by the marked points. The grey shaded area is the region where  $K^*>1$  i.e. where biofilm processes are rate limiting.  $K^*$  at the experimental conditions lied in this region, excluding a substantial boundary layer retardation.

The model predicted  $\text{NH}_4^+$  concentration profile in the biofilm indicated less than 14% decrease in  $\text{NH}_4^+$  concentration from the biofilm-bulk interface to the sand substratum, suggesting that biofilm diffusion was not rate limiting. Nitrification was therefore limited by biological reaction.



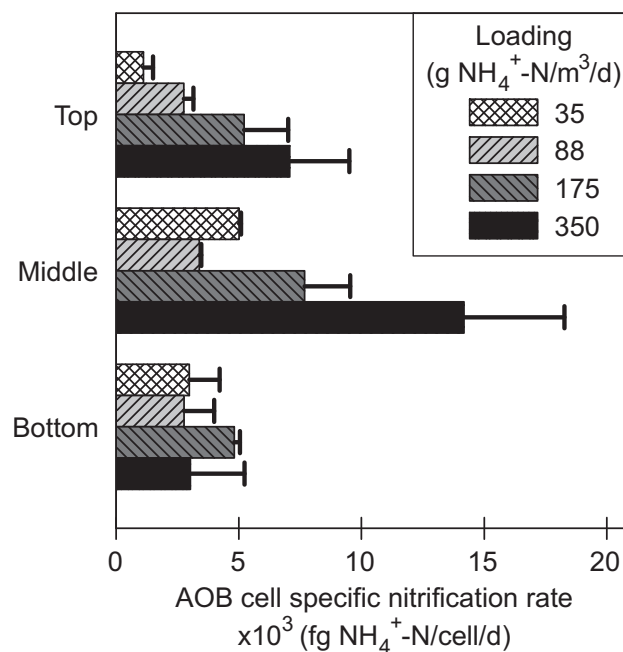


**Figure 15.** Evolution of  $K^*$  within a range of hypothetical boundary layer thicknesses in the three investigated depth layers. Marked points indicate the calculated  $K^*$  at the actual experimental conditions

Investigations by Lee et al. in pilot-scale columns that mimicked the full-scale performance at Islevbro waterworks, confirmed that nitrification was not mass transfer limited. This conclusion was based on similar removal rates observed in two columns that were operated at the same loading, but at different influent  $\text{NH}_4^+$  concentration and flowrate combinations (Lee et al., 2013). Contrarily, Lopato et al., 2013 suggested that nitrification was dominated by the pore velocity at different flowrates, implying that external mass transfer limitations were significant. However, kinetic analysis in this study was based on hydraulic patterns and depth  $\text{NH}_4^+$  profiles, therefore describing only the decrease of  $\text{NH}_4^+$  with depth and not the actual microbial kinetics.

#### 4.4. Cell specific nitrification activity and physiology of active nitrifying species

Cell specific activity was calculated in the columns for a range of loading conditions by normalizing the observed  $\text{NH}_4^+$  removal rates with the AOB cell abundance as explained in details in Tatari et al., **II**. The cell specific activity increased with loading in the top and middle layers, without reaching a maximum value within the range of tested conditions (Figure 16). Instead, the cell specific activity in the bottom layer already reached its maximum at the lowest loading condition (Figure 16). This maximum cell specific activity in the bottom was lower than the observed activities in top and middle, indicating that maximum cell specific activity varied with depth. Ultimately, this suggests the action of AOB types with different growth physiologies at the three depths.



**Figure 16.** AOB cell specific activity at all loading conditions in the bench-scale columns. Error bars represent the error calculated by propagation of the removal rate and AOB abundance standard deviations (Tatari et al., **II**)

The estimated cell specific activities agreed with values reported for AOB from previous studies with pure *Nitrosomonas*, *Nitrosolobus* and *Nitrosospira* cultures (Table 8). AOA cell specific activity in enriched marine or soil sediments on the other hand has been reported up to 2 orders of magnitude lower as indicated in Table 8.

**Table 8.** Literature reported cell-specific activities for AOB (pure cultures) and AOA (enriched cultures). Please note that AOB values are reported in different orders of magnitude

AOB		AOA	
Cell specific activity $\times 10^3$ (fg $\text{NH}_4^+$ -N/cell/d)	Reference	Cell specific activity (fg $\text{NH}_4^+$ -N/cell/d)	Reference
1.3-7.7	Prosser and Nicol, 2012	34	Kim et al., 2012
0.4-2.4	Keen and Prosser, 1987	190	Prosser and Nicol, 2012
0.3-11	Glover, 1985	35	Jung et al., 2011
7.7	Belser and Schmidt, 1980	28	Santoro and Casciotti, 2011
0.7-1.7	Remacle and Delaval, 1978	72	Park et al., 2010
		28-56	Wuchter et al., 2006
		96	Konnecke et al., 2005

#### 4.5. Selective inhibition of $\text{NH}_4^+$ and $\text{NO}_2^-$ oxidation-challenges with the use of ATU and $\text{ClO}_3^-$

Oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  (nitrification) and of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (nitrification) are linked and cannot be investigated separately unless they are uncoupled by specific inhibition. Specific inhibition investigations can seek to identify the active nitrifying microorganisms in each nitrification step and are commonly used to assess the extent of micropollutants' biological and physico-chemical degradation (Shi et al., 2004; Batt et al., 2006; Zhou and Oleszkiewicz, 2010; Falas et al., 2012). Chemical compounds are typically used to inhibit the action of a specific functional gene and block the associated function (McCarty, 1999).

Allylthiourea (ATU) is known to inhibit nitrification, while chlorate ( $\text{ClO}_3^-$ ) has been used to selectively inhibit nitrification. Inhibition by these compounds has been investigated in pure cultures, marine sediments, soils, activated sludge and BOD tests but not in filter material from rapid sand filters.

In the present work, we used ATU to investigate nitrification inhibition in filter material from the Islvebro after-filter. Our results indicated nearly complete inhibition of nitrification activity with addition of 0.1-0.5 mM ATU (Tatari et al., **III**), agreeing with previous findings reporting AOB inhibition at 0.08 mM ATU (Table 9). AOA oxidation instead, requires higher ATU concentrations, up to 0.86 mM to be significantly inhibited as reported from literature (Table 9). The inhibition observed in the present work is consistent with the evidence that AOB were the active nitrifying microorganisms in the columns (section 4.1), as this ATU concentration is too low to inhibit archaeal  $\text{NH}_4^+$  oxidation (Hatzenpichler et al., 2008; Santoro and Casciotti, 2011; Lehtovirta-Morley et al., 2013).

During these experiments, we experienced analytical interference of ATU with the indophenol blue method used to quantify  $\text{NH}_4^+$ . Analytical interference resulted in underestimation of  $\text{NH}_4^+$  concentration when ATU was present (Tatari et al. **III**). We propose that ATU possibly consumed the compound reacting with the coloring agent of the indophenol blue reaction, yielding less or no color during the spectrophotometric measurements (Tatari et al., **III**). We therefore suggest the use of alternative  $\text{NH}_4^+$  quantification methods in studies employing ATU as e.g. flow injection, unless the interference of ATU with colorimetric assays can be excluded.

**Table 9.** Overview of ATU inhibitory effect in pure cultures and environmental systems

ATU			
Concentration (mM)	Environment	% Nitritation inhibition	Reference
0.01-0.1	<i>Nitrosotalea devanterra</i> (AOA)	29-85	Lehtovirta-Morley et al., 2013
0.1-1 <sup>a</sup>	Soil microcosms	0	
0.086-0.86	CN25 (AOA)	58-100	Santoro and Casciotti, 2011
0.1	Soil slurry	53-95	Taylor et al., 2010
0.1	AOA enriched culture	Partial <sup>b</sup>	Hatzenpichler et al., 2008
0.086	AOB enriched culture	99	Ginestet et al., 1998
0.08	Lake sediments	98	Hall, 1984
0.036	BOD tests	100	Young, 1973
0.1	Rapid sand filter	87	Tatari et al., III
0.5	Rapid sand filter	96	

<sup>a</sup>ATU concentration in soil is expressed in  $\mu\text{mol/g}$  soil, <sup>b</sup>Exact % not reported

$\text{ClO}_3^-$  at 1-10 mM concentration did not inhibit nitrification, but instead blocked nitrification to nearly 85% (Table 10). However, when nitrification was isolated by feeding the experiments with  $\text{NO}_2^-$ ,  $\text{ClO}_3^-$  inhibited nitrification to a maximum of 71% (Table 10). Most studies reported in Table 10, observed nitrification inhibition in pure or enriched NOB cultures, where no nitrification was taking place. However, Hynes and Knowles experienced partial nitrification inhibition in soil slurries by  $\text{ClO}_3^-$ , that was attributed to the reduction of  $\text{ClO}_3^-$  to  $\text{ClO}_2^-$  and subsequent inhibition of nitrification by  $\text{ClO}_2^-$ . From their study, it was suggested that  $\text{ClO}_3^-$  selectivity for  $\text{NO}_2^-$  oxidation depends on the densities of nitrifying and other microorganisms that can reduce  $\text{ClO}_3^-$  to  $\text{ClO}_2^-$ , and on environmental parameters (absorption etc.) that determine  $\text{ClO}_2^-$  final bulk concentration (Hynes and Knowles, 1983).

This can potentially explain the nitrification inhibition we observed by  $\text{ClO}_3^-$  in the column, but cannot explain why nitrification was not inhibited. No nitrification inhibition by  $\text{ClO}_3^-$  was surprising and has not been previously observed. Yet, this suggests that the use of  $\text{ClO}_3^-$  to selectively inhibit nitrification in rapid sand filters is not suitable.

**Table 10.** Overview of  $\text{ClO}_3^-$  inhibitory effect in pure cultures and environmental systems

$\text{ClO}_3^-$				
Concentration (mM)	Environment	% Nitrification inhibition	% Nitrification inhibition	Reference
1-10	Aerobic granular sludge	0-26	100	Xu et al, 2011
10	<i>N. europaea</i>	0	-	Hynes and Knowles, 1983
10	Nitrobacter	-	100 <sup>b</sup>	
10	<i>N. europaea</i> and <i>Nitrobacter</i> mixed culture	60-100	-	
3-10	Nitrobacter	-	100	Belser and Mays, 1980
3-10	<i>N. europaea</i> , WH-2, D41, <i>Nitrosolobus</i> Fargo, <i>Nitrosospira</i> AV (AOB pure cultures)	<18	-	
10	Soil slurry	0	42-58	
4.2-17	Enriched <i>Nitrobacter</i>	-	Partial <sup>a</sup>	Less and Simpson, 1957
1-10 <sup>c</sup>	Rapid sand filter	80-85	2-6 <sup>d</sup>	Tatari et al., III
10-20 <sup>e</sup>	Rapid sand filter	-	67-71	

<sup>a</sup>Exact % not reported, <sup>b</sup>6 h lag time before complete inhibition was observed, <sup>c</sup> $\text{NH}_4^+$  substrate,

<sup>d</sup>Reported values are rounded, <sup>e</sup> $\text{NO}_2^-$  substrate



## 5. Conclusions

This PhD project investigated nitrification in rapid sand filters to gain insight into process biokinetics and the parameters affecting it.

The main achievements and conclusions are:

- A bench-scale assay was developed to quantify site-specific nitrification activity and to observe the biokinetic behavior at different loading conditions. The novelty of the assay consisted in employing a packed column set-up that maintains the biomass attached to the filter material, reproducing growth at full-scale filter conditions
- Nitrification in the investigated filter took place only in the top 0.20 m, while the remaining 0.50 m was inactive since it was not exposed to detectable  $\text{NH}_4^+$  loading. However, filter layers below 0.20 m were active when exposed to substantial  $\text{NH}_4^+$  loadings in the bench-scale columns
- Mineral coating on the filter material changed the bulk density and made the sand grains lighter, hence determining their depth distribution after backwashing
- Mineral coating was positively correlated with nitrification activity, suggesting that high internal porosity provides larger surface area per bulk filter material volume
- AOA abundance in the filter was comparable with AOB. However, during the column experiments we observed only increase of AOB cell numbers, while AOA abundance remained unchanged. This suggested that AOB were the main active nitrifying species at the lab-scale loading conditions
- Nitrification kinetics and response to transient loading up-shifts was stratified with depth. Filter layers above 0.30 m showed an up-shift capacity in  $\text{NH}_4^+$  oxidation, expressed by an increase of the maximum growth rate at these conditions. The deepest investigated depth layer (0.35-0.50 m) did not exhibit any up-shift capacity at increased loading levels.
- AOB physiology appeared to stratify with depth, as maximum cell specific activity varied with depth. Stratification of active nitrifiers was consistent with the different model estimated nitrification kinetic parameters (half saturation concentrations and maximum growth rates)



- Nitritation activity was successfully inhibited by 0.1 mM ATU, although we experienced interference of the compound with the indophenol blue method used to quantify  $\text{NH}_4^+$ .  $\text{ClO}_3^-$  at 1-10 mM did not inhibit nitrataion but only nitritation, conflicting with its widespread use as a selective nitrataion inhibitor

## 6. Practical considerations & perspectives

The knowledge obtained from this study generates practical considerations for filter design and operation, and raises new questions for further research as discussed in this section.

A large part of this work used site-specific investigations to focus on stratification patterns in rapid sand filters. Strong stratification observed in the investigated filter was explained by the presence of mineral coating, which determined the depth distribution of the sand grains. Ultimately, stratification in a filter indicates that backwashing is not always efficient in mixing and re-distributing the filter material. Stratification in a filter may or may not be desired, depending on the influent water composition and on the effluent water quality requirements.

In general, stratification leads to a less efficient use of the filter volume, since deeper filter layers have a lower nitrification activity or are even inactive. In the latter case, a large fraction of the filter volume is not utilized for nitrification. However, this may stimulate other processes such as  $\text{Mn}^{+2}$  removal, which is believed to take place after all  $\text{NH}_4^+$  has been completely oxidized to  $\text{NO}_3^-$ . This means that filters treating water with significant  $\text{NH}_4^+$  and  $\text{Mn}^{+2}$  contents may intentionally aim to develop distinct depth regions where each compound is removed. Additionally, stratification may select for dominant active nitrifiers with different affinities towards  $\text{NH}_4^+$ . This can be an important consideration if the effluent water is required to comply with stringent guideline limits. Nitrifiers with low affinities remove most  $\text{NH}_4^+$  loading in a filter but may be inefficient at low  $\text{NH}_4^+$  concentrations and result in effluent that exceeds the guideline limits. On the other hand, if removal to such low levels is not necessary and  $\text{Mn}^{+2}$  content is low, homogeneous distribution of nitrification activity can allow the filter to operate more efficiently at higher loadings. However, it is important to determine a safe operational loading window that aims both at the efficient use of the filter volume and at providing a safety margin for peak loadings.

Mineral coating positively correlated with nitrification activity, indicating larger area per unit filter volume. If surface area is limiting for nitrification activity, then the use of other filter media types such as expanded clays or activated carbon can provide large internal porosity and surface areas. Although expanded clays have already replaced traditional quartz sand in several newly renovated

waterworks, their properties and the link with nitrification activity needs to be further investigated to justify their high cost.

Microbial communities in rapid sand filters are also a large unexplored interest area for filter optimization. Although in the present work we detected the presence of AOB and AOA, the link between the specific microbial types and the activity observed needs to be further investigated. Detailed knowledge on AOA and AOB role and the physiology of these different microbial types can suggest a link between the presence of certain microorganisms and nitrification activity.

## 7. References

- AWWA. 1999. Water quality & treatment. 5<sup>th</sup> edition, McGraw-Hill.
- Batt AL, Kim S, Aga DS. 2006. Enhanced biodegradation of iopromide and trimethoprim in nitrifying activated sludge. *Environmental Science & Technology* 40:7367-7373.
- Belser LW, Mays EL. 1980. Specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. *Applied and Environmental Microbiology* 39:505-510.
- Belser LW, Schmidt EL. 1980. Growth and oxidation kinetics of 3 genera of ammonia oxidizing nitrifiers. *FEMS Microbiology Letters* 7:213-216.
- Boltz JP, Morgenroth E, Brockmann D, Bott C, Gellner WJ, Vanrolleghem PA. 2011. Systematic evaluation of biofilm models for engineering practice: components and critical assumptions. *Water Science and Technology* 64:930-944.
- Boltz JP, Morgenroth E, Brockmann D, Daigger GT, Henze M, Rittmann B, Sørensen KH, Takács I, Vanrolleghem PA, van Loosdrecht M. 2013. A biofilm reactor model calibration framework. 9<sup>th</sup> International Conference on Biofilm Reactors, Paris.
- Bray R, Olanczuk-Neyman K. 2001. The influence of changes in groundwater composition on the efficiency of manganese and ammonia nitrogen removal on mature quartz sand filtering beds. *Water Science and Technology: Water Supply* 1:91-98.
- Chandran K, Smets BF. 2001. Estimating biomass yield coefficients for autotrophic ammonia and nitrite oxidation from batch respirograms. *Water Research* 35:3153-3156.
- Chu CW, Lu CY, Lee CM. 2005. Effects of inorganic nutrients on the regrowth of heterotrophic bacteria in drinking water distribution systems. *Journal of Environmental Management* 74:255-263.
- Council Directive 98/93/EC. 1998. Quality of Water Intended for Human Consumption. Annex I, Part C.
- Crittenden JC, Trussell RR, Hand DW, Howe KJ, Tchobanoglous G. 2005. Water treatment. Principles and design. New Jersey, John Wiley and sons Inc.
- de Vet W, Dinkla IJT, Muyzer G, Rietveld LC, van Loosdrecht MCM. 2009. Molecular characterization of microbial populations in groundwater sources and sand filters for drinking water production. *Water Research* 43:182-194.
- Eberling B. 2002. Grundvandskemi. In: Vandforsyning, 2<sup>nd</sup> edition.
- Falas P, Andersen HR, Ledin A, Jansen JL. 2012. Impact of solid retention time and nitrification capacity on the ability of activated sludge to remove pharmaceuticals. *Environmental Technology* 33:865-872.
- Ginestet P, Audic JM, Urbain V, Block JC. 1998. Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. *Applied and Environmental Microbiology* 64:2266-2268.

- Glover HE. 1985. The relationship between inorganic nitrogen oxidation and organic carbon production in batch and chemost cultures of marine nitrifying bacteria. *Archives of Microbiology* 142:45-50.
- Hall GH. 1984. Measurement of nitrification rates in lake sediments: comparison of the nitrification inhibitors nitrapyrin and allylthiourea. *Microbial Ecology* 10:25-36.
- Hatzenpichler R, Lebedeva EV, Spieck E, Stoecker K, Richter A, Daims H, Wagner M. 2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proceedings of the National Academy of Sciences of the United States of America* 105:2134-2139.
- Hu PY, Hsieh YH, Chen JC, Chang CY. 2004. Characteristics of manganese-coated sand using SEM and EDAX analysis. *Journal of Colloid and Interface Science* 272:308-313.
- Hynes RK, Knowles R. 1983. Inhibition of chemoautotrophic nitrification by sodium chlorate and sodium chlorite: a reexamination. *Applied and Environmental Microbiology* 45:1178-1182.
- IWA. 2010. International statistics for water services. Information every manager should know about, Montreal.
- Jung MY, Park SJ, Min D, Kim JS, Rijpstra WIC, Damste JSS, Kim GJ, Madsen EL, Rhee SK. 2011. Enrichment and Characterization of an Autotrophic Ammonia-Oxidizing Archaeon of Mesophilic Crenarchaeal Group I.1a from an Agricultural Soil. *Applied and Environmental Microbiology* 77:8635-8647.
- Jupiter. 2013. National geological surveys for Denmark and Greenland.  
<http://data.geus.dk/JupiterWWW/index.jsp>
- Keen GA, Prosser JL. 1987. Steady-state and transient growth of autotrophic nitrifying bacteria. *Archives of Microbiology* 147:73-79.
- Kihn A, Laurent P, Servais P. 2000. Measurement of potential activity of fixed nitrifying bacteria in biological filters used in drinking water production. *Journal of Industrial Microbiology & Biotechnology* 24:161-166.
- Kim JG, Jung MY, Park SJ, Rijpstra WIC, Damste JSS, Madsen EL, Min D, Kim JS, Kim GJ, Rhee SK. 2012. Cultivation of a highly enriched ammonia-oxidizing archaeon of thaumarchaeotal group I.1b from an agricultural soil. *Environmental Microbiology* 14:1528-1543.
- Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437:543-546.
- Lawler DF, Nason JA. 2006. Granular media filtration: old process, new thoughts. *Water Science and Technology* 53:1-7.
- Lee CO, Boe-Hansen R, Musovic S, Smets BF, Albrechtsen H-J, Binning PJ. 2013. Effects of dynamic operating conditions on nitrification in biological rapid sand filters for drinking water treatment. Submitted to *Water Research*.
- Lees H, Simpson JR. 1957. The biochemistry of the nitrifying organisms. 5. Nitrite oxidation by *Nitrobacter*. *Biochemical Journal* 65:297-305.

- Lehtovirta-Morley LE, Verhamme DT, Nicol GW, Prosser JI. 2013. Effect of nitrification inhibitors on the growth and activity of *Nitrosotalea devanattera* in culture and soil. *Soil Biology & Biochemistry* 62:129-133.
- Lopato L. 2011. Granular filters for water treatment: heterogeneity and diagnostic tools. Kgs. Lyngby, Technical University of Denmark.
- Lopato L, Binning PJ, Arvin E. 2012. Review of diagnostic tools to investigate the physical state of rapid granular filters. *Journal of Water Supply Research and Technology-Aqua* 61:123-141.
- Lopato L, Rottgers N, Binning PJ, Arvin E. 2013. Heterogeneous Nitrification in a Full-Scale Rapid Sand Filter Treating Groundwater. *Journal of Environmental Engineering-Asce* 139:375-384.
- Lytle DA, Sorg TJ, Wang LL, Muhlen C, Rahrig M, French K. 2007. Biological nitrification in a full-scale and pilot-scale iron removal drinking water treatment plant. *Journal of Water Supply Research and Technology-Aqua* 56:125-136.
- Madoni P, Davoli D, Cavagnoli G, Cucchi A, Pedroni M, Rossi F. 2000. Microfauna and filamentous microflora in biological filters for tap water production. *Water Research* 34:3561-3572.
- Madoni P, Davoli D, Fontani N, Cucchi A, Rossi F. 2001. Spatial distribution of microorganisms and measurements of oxygen uptake rate and ammonia uptake rate activity in a drinking water biofilter. *Environmental Technology* 22:455-462.
- Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* 461:976-979.
- Martens-Habbena W, Stahl DA. 2011. Nitrogen metabolism and kinetics of Ammonia-Oxidizing Archaea. *Methods in Enzymology* 496:465-487.
- McCarty GW. 1999. Modes of action of nitrification inhibitors. *Biology and Fertility of Soils* 29:1-9.
- Metcalf & Eddy. 2004. Wastewater engineering. Treatment and reuse, 4<sup>th</sup> edition, John Wiley & sons Inc.
- Miljøministeriet. 2011. Bekendtgørelse om vandkvalitet og tilsyn med vandforsyningsanlæg 1024 from 31/10/2011.
- Mouchet P. 1992. From conventional to biological removal of iron and manganese in France. *Journal American Water Works Association* 84:158-167.
- Park BJ, Park SJ, Yoon DN, Schouten S, Damste JSS, Rhee SK. 2010. Cultivation of Autotrophic Ammonia-Oxidizing Archaea from Marine Sediments in Coculture with Sulfur-Oxidizing Bacteria. *Applied and Environmental Microbiology* 76:7575-7587.
- Pedersen HD, Postma D, Jakobsen R, Larsen O. 2005. Fast transformation of iron oxyhydroxides by the catalytic action of aqueous Fe(II). *Geochimica Et Cosmochimica Acta* 69:3967-3977.
- Prosser JI. 1989. Autotrophic nitrification in bacteria. *Advances in Microbial Physiology* 30:125-181.

- Prosser JI, Nicol GW. 2012. Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. *Trends in Microbiology* 20:523-531.
- Queinnec I, Ochoa JC, Wouwer AV, Paul E. 2006. Development and calibration of a nitrification PDE model based on experimental data issued from biofilter treating drinking water. *Biotechnology and Bioengineering* 94:209-222.
- Remacle J, DeLaval J. 1978. *Microbiology*. Washington, D.C., American Society for Microbiology.
- Rittmann B, McCarty P. 2001. *Environmental Biotechnology: Principles and Applications*. McGraw-Hill.
- Rittmann BE, Snoeyink VL. 1984. Achieving biologically stable drinking water. *Journal American Water Works Association* 76:106-114.
- Sahabi DM, Takeda M, Suzuki I, Koizumi J. 2009. Removal of  $Mn^{+2}$  from water by "aged" biofilter media: The role of catalytic oxides layers. *Journal of Bioscience and Bioengineering* 107:151-157.
- Santoro AE, Casciotti KL. 2011. Enrichment and characterization of ammonia-oxidizing archaea from the open ocean: phylogeny, physiology and stable isotope fractionation. *Isme Journal* 5:1796-1808.
- Schwartz T, Jungfer C, Heissler S, Friedrich F, Faubel W, Obst U. 2009. Combined use of molecular biology taxonomy, Raman spectrometry, and ESEM imaging to study natural biofilms grown on filter materials at waterworks. *Chemosphere* 77:249-257.
- Sharma SK, Petrusevski B, Schippers JC. 2005. Biological iron removal from groundwater: a review. *Journal of Water Supply Research and Technology-Aqua* 54:239-247.
- Shi J, Fujisawa S, Nakai S, Hosomi M. 2004. Biodegradation of natural and synthetic estrogens by nitrifying activated sludge and ammonia-oxidizing bacterium *Nitrosomonas europaea*. *Water Research* 38:2323-2330.
- Stembal T, Markic M, Ribicic N, Briski F, Sipos L. 2005. Removal of ammonia, iron and manganese from groundwaters of northern Croatia - pilot plant studies. *Process Biochemistry* 40:327-335.
- Stumm W, J.J. M. 1996. *Aquatic chemistry*, 3<sup>rd</sup> edition, John Wiley & sons Inc.
- Taylor AE, Zeglin LH, Dooley S, Myrold DD, Bottomley PJ. 2010. Evidence for Different Contributions of Archaea and Bacteria to the Ammonia-Oxidizing Potential of Diverse Oregon Soils. *Applied and Environmental Microbiology* 76:7691-7698.
- Tekerlekopoulou AG, Papazafiris PG, Vayenas DV. 2012. Effect of environmental and operating conditions on a full-scale trickling filter for well water treatment. *Desalination and Water Treatment* 39:228-234.
- Uhl W, Gimbel R. 2000. Dynamic modeling of ammonia removal at low temperatures in drinking water rapid filters. *Water Science and Technology* 41:199-206.
- Vandcenter Syd. 2013. <http://www.vandcenter.dk/viden/drikkevand>.
- Vandenabeele J, Debeer D, Germonpre R, Verstraete W. 1992. Manganese oxidation by microbial consortia from sand filters. *Microbial Ecology* 24:91-108.

- Vandforsyning. 2002. Ingeniøren bøger, 2<sup>nd</sup> edition.
- White CP, DeBry RW, Lytle DA. 2012. Microbial Survey of a Full-Scale, Biologically Active Filter for Treatment of Drinking Water. *Applied and Environmental Microbiology* 78:6390-6394.
- Winther L, Jørgen Linde J, Winther H. 2010. Vandforsyningsteknik. Kgs. Lyngby.
- Wolthoorn A, Temminghoff EJM, van Riemsdijk WH. 2004. Effect of synthetic iron colloids on the microbiological  $\text{NH}_4^+$  removal process during groundwater purification. *Water Research* 38:1884-1892.
- Wuchter C, Abbas B, Coolen MJL, Herfort L, van Bleijswijk J, Timmers P, Strous M, Teira E, Herndl GJ, Middelburg JJ, Schouten S, Damste JSS. 2006. Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences of the United States of America* 103:12317-12322.
- Xu GJ, Xu XC, Yang FL, Liu ST. 2011. Selective inhibition of nitrite oxidation by chlorate dosing in aerobic granules. *Journal of Hazardous Materials* 185:249-254.
- Young JC. 1973. Chemical methods for nitrification control. *Journal Water Pollution Control Federation* 45:637-646.
- Zhang Y, Love N, Edwards M. 2009. Nitrification in Drinking Water Systems. *Critical Reviews in Environmental Science and Technology* 39:153-208.
- Zhou XK, Oleszkiewicz JA. 2010. Biodegradation of oestrogens in nitrifying activated sludge. *Environmental Technology* 31:1263-1269.
- Zhu IX, Getting T, Bruce D. 2010. Review of biologically active filters in drinking water applications. *Journal American Water Works Association* 102:67-77.





## 8. Papers

- I.** Tatari, K., Smets, B. F., Albrechtsen H.-J., 2013. A novel bench-scale column assay to investigate site-specific nitrification biokinetics in biological rapid sand filters. *Water Research* 2013, 47, (16), 6380-6387.
- II.** Tatari, K. Musovic, S., Smets, B. F., Albrechtsen, H.-J. Strong stratification in nitrifier activity, density and type in rapid sand filters treating drinking water. Submitted to *Environmental Science & Technology*.
- III.** Tatari, K., Gülay, A., Thamdrup, B., Albrechtsen, H.-J., Smets, B. F. Use and challenges of specific nitrification inhibitors applied to groundwater treatment biofilters. Submitted to *Water Research*.
- IV.** Gülay, A., Tatari, K., Musovic, S., Mateiu, R. V., Albrechtsen, H.-J., Smets, B. F. Internal mineral porosity supports microbial activity in stratified rapid sand filters for drinking water treatment. In preparation for *Environmental Microbiology*.

In this online version of the thesis, the papers are not included but can be obtained from electronic article databases e.g. via [www.orbit.dtu.dk](http://www.orbit.dtu.dk) or on request from:

DTU Environment  
Technical University of Denmark  
Miljøvej, Building 113  
2800 Kgs. Lyngby  
Denmark

[reception@env.dtu.dk](mailto:reception@env.dtu.dk)





The Department of Environmental Engineering (DTU Environment) conducts science-based engineering research within four sections:

Water Resources Engineering, Urban Water Engineering,  
Residual Resource Engineering and Environmental Chemistry & Microbiology.

The department dates back to 1865, when Ludvig August Colding, the founder of the department, gave the first lecture on sanitary engineering as response to the cholera epidemics in Copenhagen in the late 1800s.

**DTU Environment**  
**Department of Environmental Engineering**  
Technical University of Denmark

Miljoevej, building 113  
2800 Kgs. Lyngby  
Denmark

Phone: +45 4525 1600  
Fax: +45 4593 2850  
e-mail: [reception@env.dtu.dk](mailto:reception@env.dtu.dk)  
[www.env.dtu.dk](http://www.env.dtu.dk)